Adetunji T Toriola

RESEARCH

Epidemiological Study of the Role of Vitamin D in the Aetiology of Ovarian Cancer

RESEARCH 47/2010

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ACADEMIC DISSERTATION

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Supervised by
Professor Matti Lehtinen
Tampere School of Public Health
University of Tampere
Finland

Reviewers
Emeritus Professor Antti Kauppila
University of Oulu
Department of Obstetrics and Gynaecology
Finland

Docent Merja Kärkkäinen
University of Helsinki
Department of Food and Environmental Sciences
Finland

Opponent
Professor Christel Lamberg-Allardt
University of Helsinki
Finland

Dedicated to the 3 Ts- Temi, Teni and Tose

A fact is a simple statement that everyone believes. It is innocent, unless found guilty. An hypothesis is a novel suggestion that no one wants to believe. It is guilty, until found effective. - Edward Teller

Abstract

Adetunji T Toriola. Epidemiological study of the role of vitamin D in the aetiology of ovarian cancer. National Institute for Health and Welfare (THL), Research 47. 126 pages. Helsinki, Finland 2010.
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Ovarian cancer is a very lethal gynaecological cancer because its symptoms are insidious and majority of the patients present with advanced stage disease. There is considerable geographic variation in the incidence and mortality of the disease. The gonadotrophin, hormonal, incessant ovulation and inflammation hypotheses have all been proposed to explain its aetiology and while epidemiological studies offer some support for aspects of each hypothesis, the contribution of other hitherto under-investigated factors cannot be discountenanced.

Ecological, experimental and dietary studies suggest that vitamin D may offer some protection against ovarian cancer but there is dearth of epidemiological studies investigating this association using the best marker for vitamin D; serum 25-hydroxyvitamin D (25-OHD). The aim of this thesis was to investigate the association between vitamin D and ovarian cancer utilizing serum 25-OHD concentrations which is the most reliable way to determine an individual's vitamin D status.

The studies in the thesis are a series of case-control studies nested within the Finnish Maternity Cohort (FMC) which is a population-based serum biorepository, maintained at -25^{o}C, containing the first trimester serum samples of almost all pregnant Finnish women since 1983. Ovarian cancer cases and suitably matched controls were identified after linkages with the nation-wide Finnish Cancer Registry (FCR) and Statistics, Finland.

The validity of our results depends to a large extent on how measurable and preserved the vitamin D metabolite, 25-OHD, is within serum samples that have been stored for many years. Therefore, in order to avoid differential misclassification in our studies, our first objective was to determine the stability of 25-OHD and androstenedione in serum samples that had been stored for up to 24 years. We observed the expected marked seasonal differences in serum 25-OHD concentrations for all the years studied. The mean serum 25-OHD levels were significantly higher in summer (44.0 nmol/L) compared to winter (33.4 nmol/L, p-value ≤ 0.001). There was no evidence to suggest systematic degradation of 25-OHD in stored sera, implying that 25-OHD is very stable in serum samples stored at-25oC for many years.

In the second study involving 201 ovarian cancer cases diagnosed within 10 years of serum sampling and 398 season, age and parity-matched controls, we observed no overall association between serum 25-OHD concentrations and ovarian cancer risk (OR 1.8, 95% CI 0.9 – 3.5 comparing lowest to highest quintile) but women with insufficient serum 25-OHD concentrations (< 75 nmol/L) appeared to be at increased risk of ovarian cancer (OR 2.7, 95% CI 1.0 – 7.9) compared to those with sufficient concentrations (≥ 75 nmol/L).

In study III, we sought to determine whether calcium and vitamin D act independently or jointly and whether each modifies the action of the other on ovarian cancer risk. Women within the highest quartile of calcium concentration had significantly reduced risk of ovarian cancer while women within the highest quartile of serum 25-OHD concentration had borderline reduced risk of ovarian cancer compared to those within the lowest. Women with sufficient serum 25-OHD levels had a significantly reduced risk of ovarian cancer compared to women with insufficient serum levels (OR 0.32, 95% CI 0.12-0.91). While calcium was independently associated with a reduced risk of ovarian cancer regardless of serum 25-OHD levels, (OR 0.41, 95% CI 0.19-087), vitamin D was independently associated with a non-significantly reduced risk of ovarian cancer (OR 0.51, 95% CI 0.29-1.05). We observed no evidence of effect modification between calcium and vitamin D with regards to ovarian cancer.

A perceived weakness in epidemiological studies of vitamin D and ovarian cancer is the use of one time serum 25-OHD measurement to indicate over time vitamin D status. In the only study so far that has measured serum 25-OHD twice, over a long period of time, among cases and controls; we found evidence to suggest that maintaining consistently high serum 25-OHD levels over many years during summer may be associated with a reduced risk of ovarian cancer. Women whose serum 25-OHD levels were above the season specific median values on both sampling occasions had a borderline reduced risk of ovarian cancer (OR 0.21 95% CI 0.05-0.99) compared to other women with consistently low or fluctuating serum 25-OHD levels. No such protective effect was however observed among women who donated their samples during winter.

Key words: ovarian cancer, vitamin D, 25-hydroxyvitamin D, calcium, epidemiology, biobank, cancer registry, prospective study, nested case-control, longitudinal

Tiivistelmä

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Johtuen oireiden salakavaluudesta useimmat munasarjasyöpätapaukset todetaan pitkälle edenneinä, ja munasarjasyöpä on hyvin tappava gynekologinen syöpä. Maantieteellisesti taudin ilmaantuvuus ja kuolleisuus tautiin vaihtelevat huomattavasti. Munasarjasyövän syyksi on esitetty gonadotropiinia, muuta hormonaalista syytä, keskeytymättömiä ovulaatiota ja tulehdusta. Vaikka jokaisen hypoteesin tueksi on esittää epidemiologista todistusaineistoa muiden, toistaiseksi vähän tutkittujen, tekijöiden osuutta ei voi sulkea pois.

Ekologisten, kokeellisten ja ravitsemustutkimusten perusteella D-vitamiini voi suojata munasarjasyövältä, mutta tutkimukset, jotka hyödyntävät parasta D-vitamiinin mittaustapaa, seerumin 25-hydroksi D (25-OHD) vitamiinimääritystä, puuttuvat. Tämän väitöskirjatutkimuksen tarkoituksena oli tutkia D-vitamiinin ja munasarjasyövän yhteyttä käyttäen hyväksi seerumin 25-OHD tasoja – luotettavinta tapaa määrittää yksilön D-vitamiinistatus.

Tässä väitöskirjassa esitettävät työt ovat sarja Äitikohorttiin (Finnish Maternity Cohort, FMC) upotettuja tapaus-verrokkitutkimuksia. FMC-seerumipankki on väestöpohjainen seerumipankki, jossa lähes kaikkien Suomessa vuodesta 1983 raskaana olleiden naisten ensimmäisen raskaus kolmanneksen seeruminäytettä säilytetään -25° C:ssa. Munasarjasyöpätapaukset ja kaltaistetut verrokit identifiointiin yhdistämällä tietoja Syöpärekisterin ja Tilastokeskuksen kanssa.

Tulostemme oikeellisuus riippuu paljolti siitä, miten hyvin D-vitamiinin 25-OHD aineenvaihduntatuote on säilynyt ja mitattavissa vuosi säilytetyissä seeruminäytteissä. Sen vuoksi, välttääksemme virheluokitusta, ensimmäinen tavoitteemme oli määrittää 25-OHD:n ja androstenedionin säilyminen seeruminäytteissä, joita oli säilytetty jopa 24 vuotta. Havaitsimme odotetut, vuodenajasta johtuvat seerumin 25-OHD tasojen erot kaikkina seurantavuosina. Keskimääräinen seerumin 25-OHD taso oli merkitsevästi korkeampi kesällä (44.0 nmol/l) talveen (33.4 nmol/l) verrattuna ($p<0.001$). Emme havainneet merkkejä systemaattisesta 25-OHD kadosta säilytetyissä seerumeissa, mikä viittaa siihen, että 25-OHD pysyy muuttumattomana useiden vuosien ajan -25° C:ssa säilytetyissä seeruminäytteissä.

Toisessa tutkimuksessa, joka koski 201 munasarjasyöpätapausta, jotka oli diagnostisoitu 10 vuoden kuluessa seeruminäytteen ottamisesta, ja 398 vuodenajan, iän ja raskauksien määrän suhteen kaltaistettua verrokkia emme havainneet yhteyttä seerumin 25-OHD pitoisuuden ja munasarjasyöpäriskin välillä (OR 1.8, 95% CI 0.9 – 3.5) vertailtaessa alimman ja ylimmän neljänneksen D-vitamiinitason omanneita tutkittavia. Naisilla, joilla oli suositusten mukaan riittämätön 25-OHD taso (<75 nmol/l) näytti kuitenkin olevan kohonnut munasarjasyövän riski (OR 2.7, 95% CI 1.0 – 7.9) verrattuna riittävät tasot (> 75 nmol/l) omanneisiin naisiin.

Kolmannessa tutkimuksessa selvitimme toimivatko kalsium ja D vitamiini riippumattomasti vai muuntelevatko ne toistensa vaikutusta munasarjasyöpäriskiin. Naisilla, jotka olivat kalsium tasojensa suhteen korkeimmassa neljänneksessä, oli merkitsevästi vähentynyt munasarjasyöpä-riski kun taas naisten, jotka olivat korkeimmassa D vitamiinineljänneksessä, munasarjasyöpä-riskin vähenemisen tilastollinen merkitsevyys jäi rajalle. Naisilla, joilla oli riittä-

vät 25-OHD tasot oli merkitsevästä alentunut munasarjasyöpäriski (OR 0.32, 95% CI 0.12 – 0.91). Kalsiumiin tilastollisesti merkitsevästi liittyvä alentunut munasarjasyöpäriski oli riippumaton seerumin 25-OHD tasoista (OR 0.41, 95% CI 0.19 – 0.87). D vitamiiniin liittyvä alentunut munasarjasyöpä-riski (OR 0.51, 95% CI 0.29 – 1.05) oli riippumaton. Emme havainneet viitteitä, että kalsium ja D vitamiini muuntelisivat toistensa vaikusta suhteessa munasarjasyöpään.

D vitamiinia ja munasarjasyöpää koskeneiden epidemiologisten tutkimusten todettu heikkous on ollut yhden seeruminäytteen käyttö arvioitaessa elimistön D vitamiinistatusta yli ajan. Toistaiseksi ainoassa tutkimuksessa, jossa seerumin 25-OHD tasoja on mitattu kaksi kertaa pitkällä aikavälillä, havaitsimme, että korkean seerumi 25-OHD tason säilyttäminen pitkän aikaa voi liittyä alentuneeseen munasarjasyöpäriskiin. Naisilla, joiden seerumin 25-OHD tasot olivat vuodenajan keskimääräisiä tasoja korkeammat molemmissa näytteissä oli alentunut riski sairastua munasarjasyöpään (OR 0.21, 95% CI 0.05 – 0.99). Tätä, tilastollisen merkitsevyyden rajalla ollutta, suojavaikutusta ei havaittu naisilla, joiden näytteet oli otettu talvella.

Table of Contents

List of original papers

This thesis is based on the publications listed below which are referenced in the text by their Roman numerals:

I. Agborsangaya C, **Toriola AT**, Grankvist K, Surcel H-M, Holl K, Tuohimaa P, Lukanova A, Lehtinen M. The effects of storage time and sampling season on the stability of serum 25-hydroxy vitamin D and androstenedione. Nutr Cancer 2010; 62:51-7

II. **Toriola AT**, Surcel H-M, Agborsangaya C, Grankvist K, Tuohimaa P, Toniolo P, Lukanova A, Pukkala E, Lehtinen M. Serum 25-Hydroxyvitamin D and the risk of ovarian cancer. Eur J Cancer 2010; 46: 364-369

III. **Toriola AT**, Surcel H-M, Agborsangaya C, Grankvist K, Luostarinen T, Lukanova A, Pukkala E, Lehtinen M. Independent and joint effects of serum 25-hydroxyvitamin D and calcium on ovarian cancer risk: a prospective nested case-control study. Eur J Cancer 2010;46;2799-2805

IV. **Toriola AT**, Agborsangaya C, Surcel H-M, Grankvist K, Pukkala E, Lukanova A, Lehtinen M. Can over time vitamin D status predict ovarian cancer risk? A longitudinal study nested within the Finnish Maternity Cohort. Submitted

Abbreviations

1α,25-$(OH)_2$D	1α,25-dihydroxyvitamin D
24R,25-$(OH)_2$D3	24,25-dihydroxyvitamin D
25-OHD	25-hydroxyvitamin D
7-DHC	7-dehydrocholesterol
ASR	Age standardized rates
BMI	Body mass index
CDK	Cyclin dependent kinase
CDKI	Cyclin dependent kinase inhibitor
CRP	C-reactive protein
DNA	Deoxyribonucleic acid
EGFR	Epidermal growth factor receptor
EOC	Epithelial ovarian cancer
FCR	Finnish Cancer Registry
FGF-23	Fibroblast growth factor 23
FMC	Finnish Maternity Cohort
GCT	Germ cell tumour
HGSC	High-grade serous carcinoma
HRT	Hormone replacement therapy
hTERT	human telomerase reverse transcriptase
IARC	International Agency for Research on Cancer
ICD	International Classification of Diseases
IGFBP3	Insulin growth factor binding protein 3
IU	International unit
LGSC	Low-grade serous carcinoma
MMR	Mismatch repair
NSAID	Non steroidal anti-inflammatory drug
OCP	Oral contraceptive pill
OR	Odds ratio
PKC	Protein kinase C
PTH	Parathyroid hormone
RANKL	Receptor activator nuclear factor-κB ligand
RIA	Radioimmunoassay
RR	Relative risk
RXR	Retinoid X receptor
SCST	Sex cord stromal tumour
SRC	Steroid receptor coactivator
SZA	Solar zenith angles
TGF- β	Transforming growth factor- β

THL	National Institute for Health and Welfare
UVB	Ultraviolet B
VDBP	Vitamin D binding protein
VDR	Vitamin D receptor
VDRE	Vitamin D response elements
WHO	World Health Organization
WT1	Wilms Tumour 1

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1 INTRODUCTION

Ovarian cancer is without doubt a very perplexing disease, and defining the aetiological risk factors has so far proved challenging. This, together with the fact that ovarian cancer symptoms are poorly characterized, prolonging its diagnosis, and makes it the most lethal gynaecological cancer.

An important insight into the likely aetiological factors is the marked geographical variations in the incidence of the disease and immigration studies which reveal that the incidence of the disease among immigrants from low-risk to high-risk areas tends to catch up with that of the high-risk area within a generation. This implies that environmental factors are very important determinants of ovarian cancer aetiology.

Among the factors which may be associated with ovarian cancer risk, the roles of sex hormones have been mostly studied because the overt disease is often associated with altered hormone states. Recent studies have, however, suggested that other pathways especially the vitamin D endocrine system may be involved in ovarian cancer risk but the potentials of these alternatives have not been thoroughly investigated. Previous studies that had delved into ovarian cancer-vitamin D link were mainly ecological and dietary with inherent weaknesses to establish a definite relationship between vitamin D and ovarian cancer.

The Finnish Maternity Cohort (FMC) biobank, with its serial serum samples, offers a very unique opportunity to investigate the relationship between pre-diagnostic vitamin D status (as measured by serum vitamin D levels) and ovarian cancer, and to determine whether factors which modify serum vitamin D concentrations affect this relationship

2 LITERATURE REVIEW

2.1 Pathology and histological classification of ovarian cancers

Ovarian cancer is broadly divided into three major groups: sex cord stromal tumours, germ cell tumours and surface epithelial stromal tumours depending on the tissue/anatomic structures from which the tumours originate (Scully and Sobin 1999, Chen et al 2003, Jaffe 2003). Each group is equally subdivided into various subtypes. Occasionally, two or more subtypes may be present in one tumour, in which case, it is called a mixed tumour and if a tumour subtype contributes more than 10% of the tumour mass, it is specified in the name (Seidman et al 2003, Soslow 2008). The surface epithelial stromal tumours are the most common group and they account for 80-90% of ovarian cancers in most industrialised countries (Chen et al 2003)

2.1.1 Sex cord-stromal tumours

These tumours arise from mesenchymal and mesonephric embryonic origins and account for about 7% of all ovarian cancers (Chen et al 2003). Because of their cellular origin, they are usually associated with endocrine abnormalities, particularly estrogenic but occasionally androgenic (Roth 2006). The major subtypes are (Roth 2006)

1. Granulosa cell tumours, with two types: a) the adult type which represents 95% of all ovarian granulosa cell tumours, usually occur in post-menopausal women and are associated with overproduction of estrogen, and b) the juvenile type, which may occur before puberty and cause precocious sexual development.
2. Thecomas. These comprise lipid containing cells similar to those of the theca interna.
3. Fibrosarcomas. These are dense, large masses made up of spindle-shaped cells with a storiform pattern.
4. Sertoli cell tumours. These tumours arise from rete ovarii and rete testis.
5. Sertoli-Leydig cell tumours. These tumours are admixture of epithelial and testicular cells and usually cause both androgenic (virilisation) and estrogenic manifestations.
6. Steroid cell tumours. These tumours comprise ovarian cancer cells that resemble steroid hormone secreting cells.

2.1.2 Germ cell tumours

These tumours arise from cells derived from the primordial germ cells and are the rarest ovarian cancers in western countries accounting for about 3% of cases (Talerman 1994, William et al 1997). They constitute a large proportion of the ovarian cancers diagnosed among children and adolescents (Chen 2003).

1. Dysgerminomas. These are the most common ovarian germ cell tumours with most cases occurring during adolescence and early adulthood.
2. Yolk sac tumours, also known as endodermal sinus tumours. The cellular architecture of yolk sac tumours is very similar to those of the primitive yolk sac. They express high levels of alpha-fetoprotein (AFP).
3. Embryonal carcinoma. These are usually large, solid tumours with haemorrhagic and necrotic areas. They also produce AFP and human chorionic gonadotrophin (HCG).
4. Choriocarcinoma. These tumours are formed by trophoblastic cells and may be non-gestational, where they are unrelated to pregnancy (majority) or gestational when they occur just after a pregnancy. HCG is the tumour marker of choriocarcinoma.
5. Teratoma. They develop from totipotential germ cells and thus contain all three germ cell layers; ectoderm, mesoderm and endoderm.

2.1.3 Surface epithelial-stromal carcinomas

These tumours account for about 90% of all ovarian cancers encountered in western populations (Scully et al 1998, Prat 2004). There are about five major subtypes which differ with respect to epidemiological and genetic risk factors, origin, molecular events during oncogenesis and response to treatment (Weiss et al 1996, Soslow 2008, Gilks and Prat 2009).

1. Serous carcinoma is by far the most common type of epithelial-stromal carcinomas. Previously, it was thought that serous carcinomas accounted for about 50% of all epithelial-stromal tumours but based on modern histotyping criteria, serous carcinomas are now thought to account for between 67.5% and 80% of all epithelial tumours (Seidman 2004). These cancers display a wide morphological spectrum but most consist of papillary, solid areas with slit-like appearances resembling a labyrinth. They overexpress p53 and WT1 (Wilms Tumour 1 suppression gene) in about 80% of tumours (Soslow 2008, Gilks and Prat 2009).

   An important advance in ovarian cancer histopathology was the realisation that serous carcinomas comprise two very distinct cancers with different underlying pathogenesis, molecular events and behaviour.

Serous carcinomas are divided into High-Grade Serous Carcinoma (HGSC) and Low-Grade Serous Carcinoma (LGSC) (Shih and Kurman 2004). HGSCs are predominant, have high mitotic rates, with more than 3-fold variations in nuclear size as compared to the LGSCs. Likewise, only HGSCs are associated with abnormalities in BRCA1 or BRCA2 and p53 genes while LGSCs are more likely to have *k-ras* or *b-raf* mutations (Soslow 2008, Gilks and Prat 2009).

2. Endometroid carcinoma. It is presently estimated that endometroid carcinomas account for about 10% of epithelial-stromal carcinomas, from the previously estimated 10-25% (Gilks and Prat 2009). This is because endometroid carcinoma shares a substantial morphologic overlap with serous carcinoma, especially HGSC, and many cases previously diagnosed as endometroid carcinomas were later found to be serous carcinomas (Gilks and Prat 2009). Endometroid carcinomas are usually associated with endometriosis (42% of cases) and endometrial carcinomas (20% of cases). The most common genetic abnormalities observed in endometroid carcinomas are somatic mutations in beta-catenin and PTEN genes. Unlike other ovarian cancers, most cases of endometroid carcinomas are FIGO stage 1 at diagnosis (Soslow 2008, Gilks and Prat 2009).

3. Mucinous carcinomas. They have mucin-rich cytoplasm, usually with mucin vacuoles. They comprise about 3% of epithelial-stromal carcinomas (Soslow 2008). Previously, ovarian metastases of extraovarian mucin-producing adenocarcinomas were erroneously classified as being of primary ovarian origin, which accounted for their higher prevalence in the past. Primary mucinous ovarian carcinomas are usually large, unilateral, confined to the ovarian and without involvement of the ovarian surface in contrast to metastases. They preferentially express cytokeratin 7 (CK7). (Soslow 2008, Gilks and Prat 2009).

4. Clear cell carcinoma. Clear cell carcinomas are made up of hobnail cells with clear cytoplasm. They are rare and a majority of them are associated with endometriosis, where they arise in endometriotic cysts (Chen et al 2003, Gilks and Prat 2009).

5. Transitional cell carcinomas. These are very rare and are made up of low-grade cells with longitudinal nuclear grooves, arranged in broad papillae. They bear a striking morphological resemblance to urothelial carcinomas (Soslow 2008).

6. Mixed epithelial carcinomas. These occur when the tumour comprises at least 2 histologically distinct subtypes, each contributing at least 10% to the tumour architecture. The most common variant is a combination of

endometroid and clear cell carcinoma since they both frequently arise within endometriosis (Soslow 2008).

2.1.3.1 Origin of Surface epithelial-stromal carcinomas

A long-held concept is that epithelial-stromal carcinomas arise from the ovarian surface epithelium (OSE), being mesothelial in origin (Ressa et al 1993, Nicosia et al 1999, Auersperg et al 1998), and subsequently undergo metaplastic transformation into different cell types (serous, endometroid, mucinous and clear cell), which are Mullerian in origin. Thereafter, they undergo neoplastic changes within the hormone rich cortical inclusion cysts of the ovarian parenchyma (Mittal et al 1993, Deligdisch et al 1995, Scully 1995, Blaustein et al 1992). Thus, histologically, the ovarian surface epithelium is very different from its supposed derivatives, viz a viz serous, endometroid, mucinous, clear cell and transitional carcinomas. According to this theory, malignant ovarian tumours can only arise after a specific cell-type (mesothelial) has completely changed its differentiation lineage (Mullerian) (Dubeau 2008). Likewise, the ovarian surface epithelium is monolayered, expresses little or no E-cadherin while many epithelial ovarian cancers express E-cadherin and exhibit complex histological differentiation patterns (Naora 2005). This seeming developmental aberration together with the fact that no ovarian carcinoma precursor lesions have been identified within the coelomic surface epithelium has opened new vistas in the characterization of the origin of epithelial carcinomas.

One of the new theories, backed by genetic and morphologic evidence proposes that some ovarian carcinomas are likely to originate from the Mullerian system (Dubeau 1999). Serous, endometroid, clear cell and mucinous carcinomas morphologically resemble the epithelia of the fallopian tube, endometrium and endocervix respectively, all of which are derived from the Mullerian ducts. At the molecular level, this theory is backed by the observation of Cheng and colleagues that serous, endometroid and mucinous carcinomas express the same HOX genes as epithelial cells from normal fallopian tube, endometrium and endocervix, respectively (Cheng et al 2005).

The HOX genes are involved in morphogenesis of different segments of the female reproductive tract and they are highly specific for the different segments (Cheng et al 2005, Dubeau 2008). With regard to some serous tumours, it is thought that normal epithelial cells from the fimbriated end of the fallopian tube may dislodge when the fimbriated end is in contact with the ovarian surface and implant on the site of rupture where ovulation occurred causing an inclusion cyst to form (Dubeau 2008, Kurman and Shih 2010). These inclusion cysts may then become malignant supporting the

earlier held notion that serous carcinomas may develop from inclusion cysts but through a process of implantation of tubal (Mullerian type) tissue rather than through metaplasia from ovarian surface epithelium (Dubeau 2008). Further evidence supporting a tubal origin for most HGSCs include the expression of PAX8, a Mullerian marker, but not calretinin, a mesothelial marker (Kurman and Shih 2010), and observations that obliteration of some parts of the Mullerian tract in the absence of ovarian ablation can protect against ovarian cancer.

While aspects of each of these theories are probably valid, none of them completely explains all aspects of ovarian carcinogenesis and the search for an all-encompassing theory to explain the origin of ovarian cancer, is far from over. This is of particular importance given the fact that the various histological subtypes of ovarian cancer have different reproductive (Titus-Ernstoff et al 2001), and hence hormonal, risk factors and the hormonal and similar milieu under which ovarian cancer develops, though have been explored in previous studies, need to be expanded further.

2.1.4 Borderline tumours of the ovary

These comprise a separate group of tumours, with similar epidemiological but different pathological characteristics to the malignant tumours (Lukanova and Kaaks 2006). Histo-pathologically, they are mainly serous, mucinous and endometroid tumours but they occur in younger age groups, present at earlier stages and have better prognosis compared to the malignant tumours (Lukanova and Kaaks 2005). Grossly, borderline tumours are similar to benign tumours but they usually have more exuberant projections within the cystic cavity in the case of serous tumours and more solid areas in the case of mucinous tumours but the solid areas are smaller, with less haemorrhagic zones compared with malignant tumours (Chen et al 2003).

Figure 1. Age standardized incidence rate (ASR) of ovarian cancer, worldwide, 2008

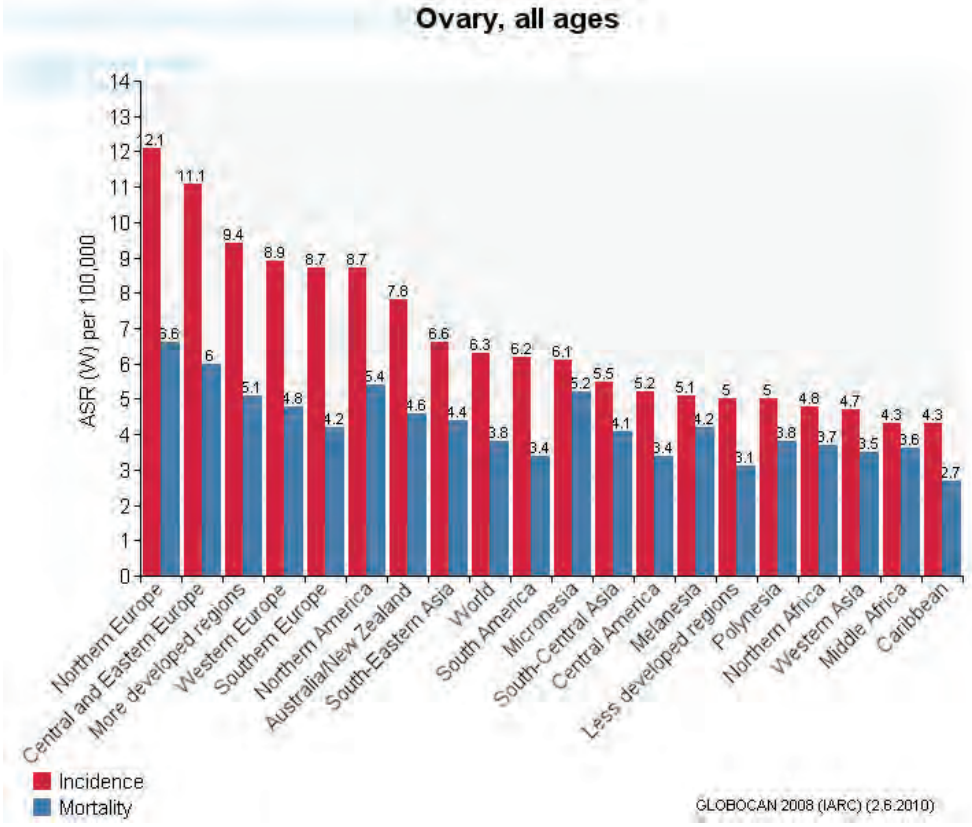
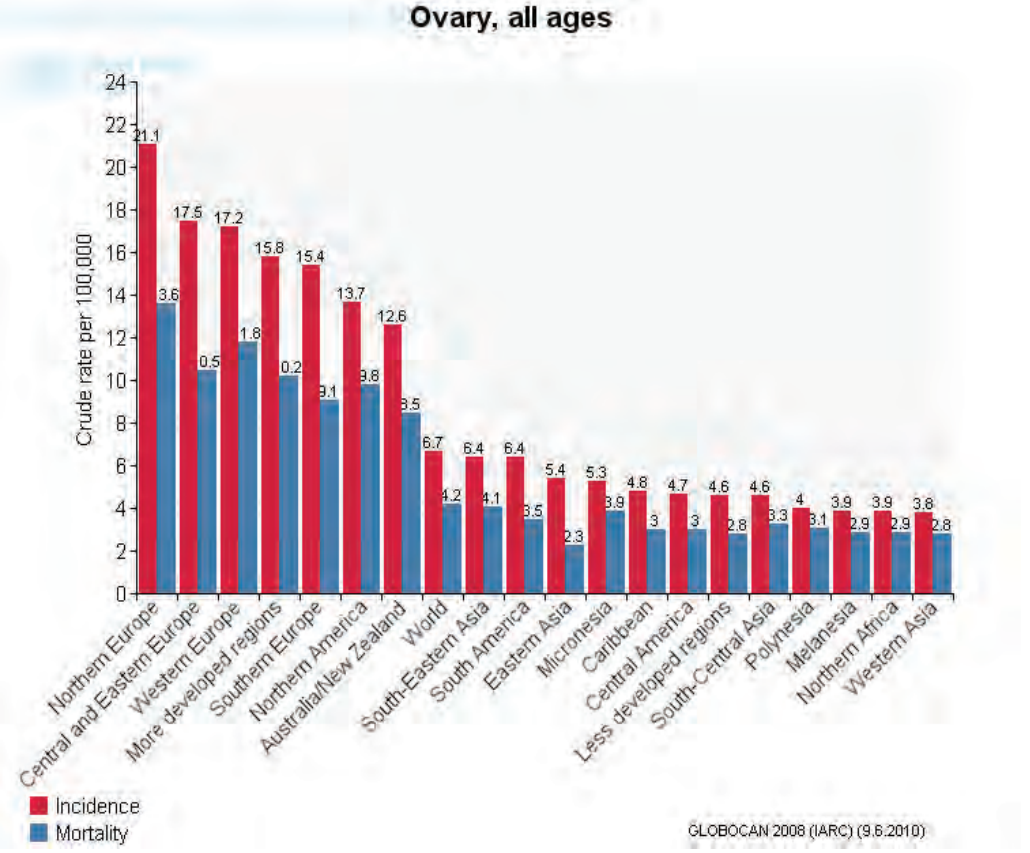


Figure 2. Crude rate of ovarian cancer, worldwide 2008



2.2 Descriptive Epidemiology of ovarian cancer

Ovarian cancer is the sixth most incident cancer (4% of cases) and the seventh most common cause of cancer death (4.2% deaths) among women worldwide (Parkin et al 2005). It is the second most common gynaecological cancer (19% and 29% of all gynaecological cancers in developing and developed countries respectively) but the most lethal (Sankaranarayanan and Ferlay 2006).

There are marked geographical differences in the incidence and mortality rates of ovarian cancer with an almost four-fold difference in rates observed between the highest and the lowest incidence regions (GLOBOCAN 2008, IARC). The highest age-standardized incident rates are observed in Northern Europe (12.1/100,000), Central and Eastern Europe (11.1/100,000) while Western and Southern Africa have the lowest age-standardized incidence rates (3.8/100,000) (GLOBOCAN 2008, IARC). Likewise, trends in

incidence and mortality rates have varied widely across the world. Incidence rates have been declining in Northern America and most parts of Northern Europe but they have been steadily increasing in some of the Central and Eastern European countries (Bray et al 2005). The increases in parts of Europe are however, more conspicuous among older women compared to younger women but a different pattern is projected worldwide. According to the latest WHO projections, a 30% increase in the annual number of new cases, worldwide, is expected by the year 2020, mostly driven by increasing incidence among women less than 65 years (GLOBOCAN 2008, IARC).

Figure 3.

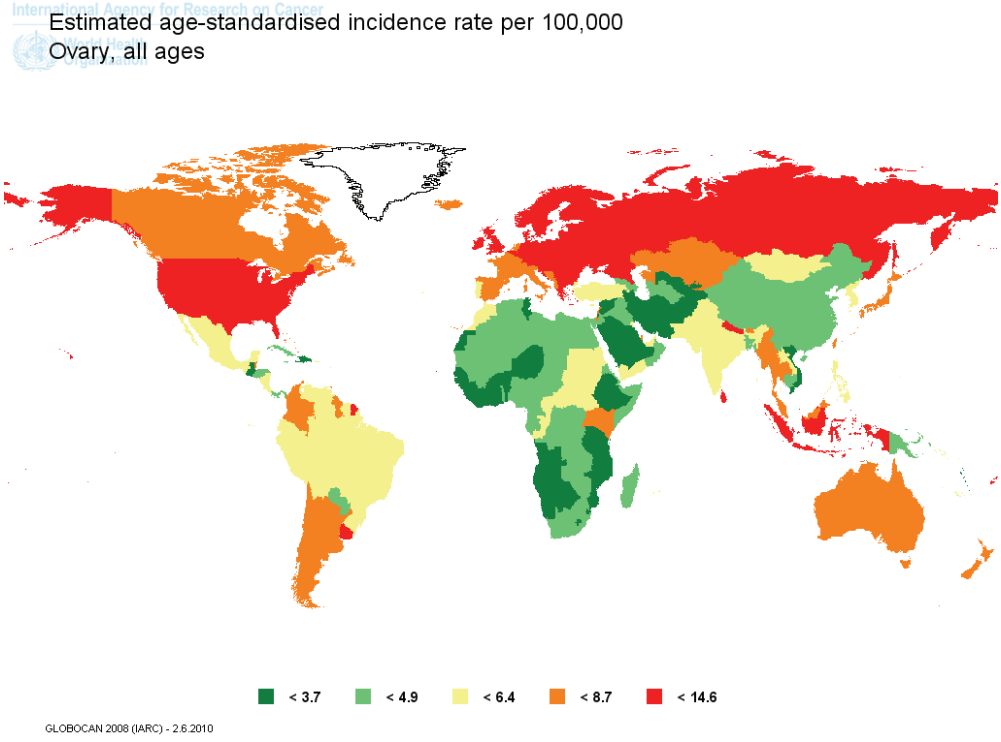
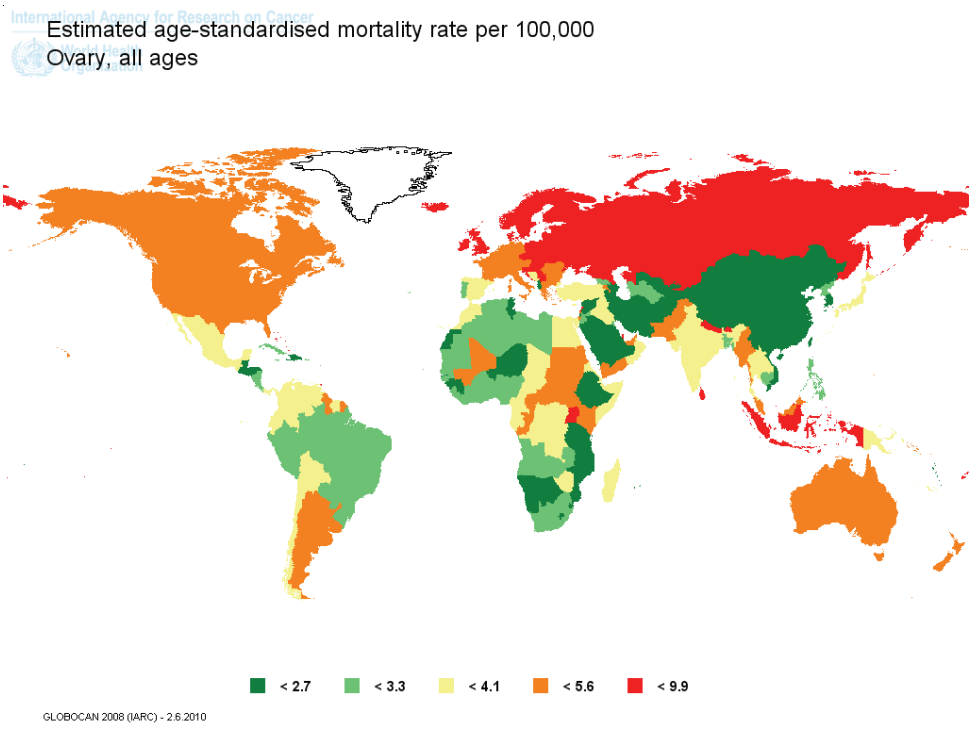


Figure 4.



2.3 Etiologic Factors

Established risk factors for ovarian cancer include age, family history of ovarian cancer, the use of hormone replacement therapy (HRT) and infertility while inflammation is a strongly suspected risk factor. On the other hand, increasing parity, use of hormonal contraception, (oral contraceptive pills, OCP), tubal ligation and hysterectomy are known to be protective while lactation is suspected to be protective (Whittemore et al 1992, Riman et al 2002, Sueblinvong and Carney 2009, Permuth-Wey and Sellers 2009).

2.3.1 Age

Incidence rates of ovarian cancer increase with age. The majority of cases (80 to 90%) are diagnosed during the peri and postmenopausal periods, the median age at diagnosis being between 58 and 65 years (Holschneider and Berek 2000). Hereditary ovarian cancer however develops at an earlier age with each successive generation (Goldberg et al 1997).

2.3.2 Family history

About 10% of ovarian cancers are hereditary (Antoniou et al 2000). Germline mutations in the high penetrance BRCA1 and BRCA2 tumour suppressor genes contribute 90% of the hereditary component. Whereas the lifetime risk of ovarian cancer in the general population is 2%, it approaches 40-65% and 20% among those with BRCA1 and BRCA2 mutations, respectively (Ford et al 1998, King et al 2003). Hereditary ovarian cancer is also associated with the Lynch Syndrome (hereditary nonpolyposis colorectal cancer) (Lynch et al 2009). This is due to mutations in the mismatch repair (MMR) genes (MLH1, MSH2, MSH6, and PMS2 genes). Carriers of these defective genes are susceptible to cancers arising in different parts of the body particularly the colorectum, ovary, endometrium, stomach and small intestine (Lynch et al 2009).

2.3.3 Hormone Replacement Therapy

Initially, the impact of hormone replacement therapy (HRT) on ovarian cancer risk was controversial. While some studies observed no increased risk of ovarian cancer associated with HRT, (Sit et al 2002, Purdie et al 1999), others did (Lacey et al 2002, Lacey et al 2006). However, the two most recent and largest studies to date have observed that not only is HRT associated with increased risk of ovarian cancer and ovarian cancer mortality, the risk increases with duration of use (Beral et al 2007, Morch et al 2009). Furthermore, the risk was evident regardless of HRT formulation, regimen, route of administration, reproductive history, previous use of oral contraceptives and socio-economic status. The million women study (Beral et al 2007) however noted that the risk did vary by tumour histology with the greatest risks observed for serous tumours. Risk among past users is similar to that of never users indicating that the risk disappears after stopping HRT use. According to the million women study, the use of HRT resulted in 1,300 and 1,000 additional ovarian cancer cases and deaths respectively in the UK between 1991 and 2009.

2.3.4 Inflammation

An association between inflammation and ovarian cancer has been suggested because factors which cause local inflammation of the ovary are associated with increased risk of ovarian cancer (Ness and Cottreau 1999). These are perineal talc use, endometriosis and pelvic inflammatory disease (Risch and Howe 1995, Brinton et al 1997, Langseth et al 2008). Likewise, gynaecological operations like tubal ligation, hysterectomy which prevent retrograde transport of inflammatory substances from the lower genital tract to the ovaries and the use of non-steroidal anti-inflammatory drugs (NSAIDs) are associated with reduced risk of the disease (Green et al 1997, Schildkraut et al 2007). Of the three prospective studies that have investigated the relationship between circulating C-reactive protein (CRP) which is an inflammatory biomarker and ovarian cancer risk, two observed increased risk of ovarian cancer among women within the highest CRP concentrations (McSorley et al 2007, Toriola et al 2010) while the third though did not observe an overall association, found that risk of ovarian cancer was higher among women with very high CRP concentrations (Lundin et al 2009).

2.3.5 Infertility

Many studies have observed a positive relationship between infertility and ovarian cancer (Rossing et al 1994, Venn et al 1995). The increased risk appears to be most pronounced among nulligravid women who have been trying to become pregnant for many years but the excess risk was not associated with the use of fertility drugs (Ness et al 2002). Among women who have been pregnant, nulliparous women, but not parous women, are at increased risk, especially those whose infertility manifested late in reproductive life. Role for specific type of infertility has not been confirmed (Ness et al 2002, Rossing et al 2004) but it is noteworthy that pelvic inflammatory disease has been associated with both tubal factor infertility and ovarian cancer.

2.3.6 Parity

One of the most consistent findings in ovarian cancer epidemiology is the protective effect conferred by full-term pregnancy. Parity reduces the risk of ovarian cancer by 30 to 70% and each additional pregnancy is estimated to confer an extra 10 to 20% reduction in the risk (Cramer et al 1983, Wittenberg J et al 1999, Adami et al 1994, Whittemore et al 1992). It is postulated that pregnancy reduces ovarian cancer risk by causing anovulation, suppressing pituitary gonadotrophins secretion and temporarily

interrupting the retrograde transport of menstrual blood flow through the fallopian tubes, hence allowing time for apoptosis. If parity protects against ovarian cancer only through these mechanisms, a similar level of protection might be associated with lactation since the same mechanisms operate during lactation. The protective effects of lactation are weaker compared to those observed with parity and not all studies have observed a protective effect (Whittemore 1992 et al, Rosenblatt et al 1993, Danforth et al 2007). Thus, the altered hormonal milieu of pregnancy is also likely to play an important role. The effects of incomplete pregnancies, whether spontaneous or induced are less clear (Cramer et al 1983, Whittemore et al 1992, Riman et al 2002).

2.3.7 Gynecological surgery

Majority of studies have observed a protective effect for tubal ligation and hysterectomy against ovarian cancer (Riman et al 2002, Hankinson et al 1993, Green et al 1997). According to some studies the protection can last up to twenty years after the surgery (Miracle-McMahill et al 1997, Green A et al 1997). It is also acknowledged that the magnitude of protection offered by tubal ligation is higher than that of hysterectomy. Among high risk women, the risk reduction may be as high as 90% (Domcheck and Rebbeck 2007). These two gynaecological procedures are believed to reduce ovarian cancer risk by preventing communication between the ovaries and the external genital tract and thus precluding the ascension of carcinogenic substances from the external genital tract to the ovaries.

2.3.8 Oral contraceptive pill

OCP use protects against ovarian cancer regardless of other known risk factors (Hankinson et al 1992, La Vecchia 2006). A recent collaborative re-analysis of 45 studies with 23,257 cases and 87,303 controls revealed that the protection offered by OCPs can last for as long as 30 years after cessation of use, even though the risk reduction became attenuated over time (Beral et al 2008). The authors concluded that the OCPs have prevented about 200,000 ovarian cancers and 100,000 ovarian cancer deaths since they were introduced a little over 50 years ago.

2.3.9 Dietary factors including vitamins

The effect of dietary habits on ovarian cancer may be direct (as viewed in terms of individual nutrients, described below) or indirect through its influence on total energy intake viz a viz obesity. Evidence is accruing that

obesity may be positively associated with risk of ovarian cancer. A recent pooled analysis of twelve studies observed that obese pre-menopausal, but not post-menopausal women had 72% increased risk of ovarian cancer compared to women with normal BMI, though BMI in early adulthood was not associated with increased risks of ovarian cancer (Schouten et al 2008).

Less proven is the impact of dietary habits and individual nutrients in ovarian cancer prevention. Fruits and vegetables contain bio-active substances that have cancer-prevention potentials. While high levels of fruits and vegetable consumption have been associated with reduced risk of some cancers, the same can not be said of ovarian cancer. A pooled analysis of cohort studies found no evidence to suggest that fruits and vegetables can offer protection against ovarian cancer (Koushik et al 2005).

Most of the prospective studies examining the associations between vitamins A, C, E and specific carotenoids have yielded negative overall results. In the Nurses Health Study with 301 invasive ovarian cancer cases, intake of vitamins A, C, E and other carotenoids from food or supplements was not associated with reduced risk of ovarian cancer (Fairfield et al 2001). The authors, however, noted that a high total intake of fruits and vegetables during adolescence was associated with a reduced risk of ovarian cancer. Similar null associations were observed in the Women's Health Initiative study, a cohort study in Canada (Thomson et al 2008, Navarro Silvera et al 2006). A pooled analysis of ten prospective studies did not observe that the major carotenoids (alpha-carotene, beta-carotene, beta-cryptoxanthin, lutein and lycopene) had any protective effect on ovarian cancer risk (Koushik et al 2006). Nevertheless, Tung K-H and colleagues observed that vitamin A and beta-carotene, but not the other anti-oxidants had modest protective effects on ovarian cancer risk, especially of the mucinous type (Tung et al 2005).

While there appears to be very weak or no inverse relationship between folate and related nutrients on ovarian cancer risk, there are suggestions that high folate intake may be protective against ovarian cancer mainly among women who consume alcohol (Larsson et al 2004, Navarro Silvera et al 2006, Tworogger et al 2006).

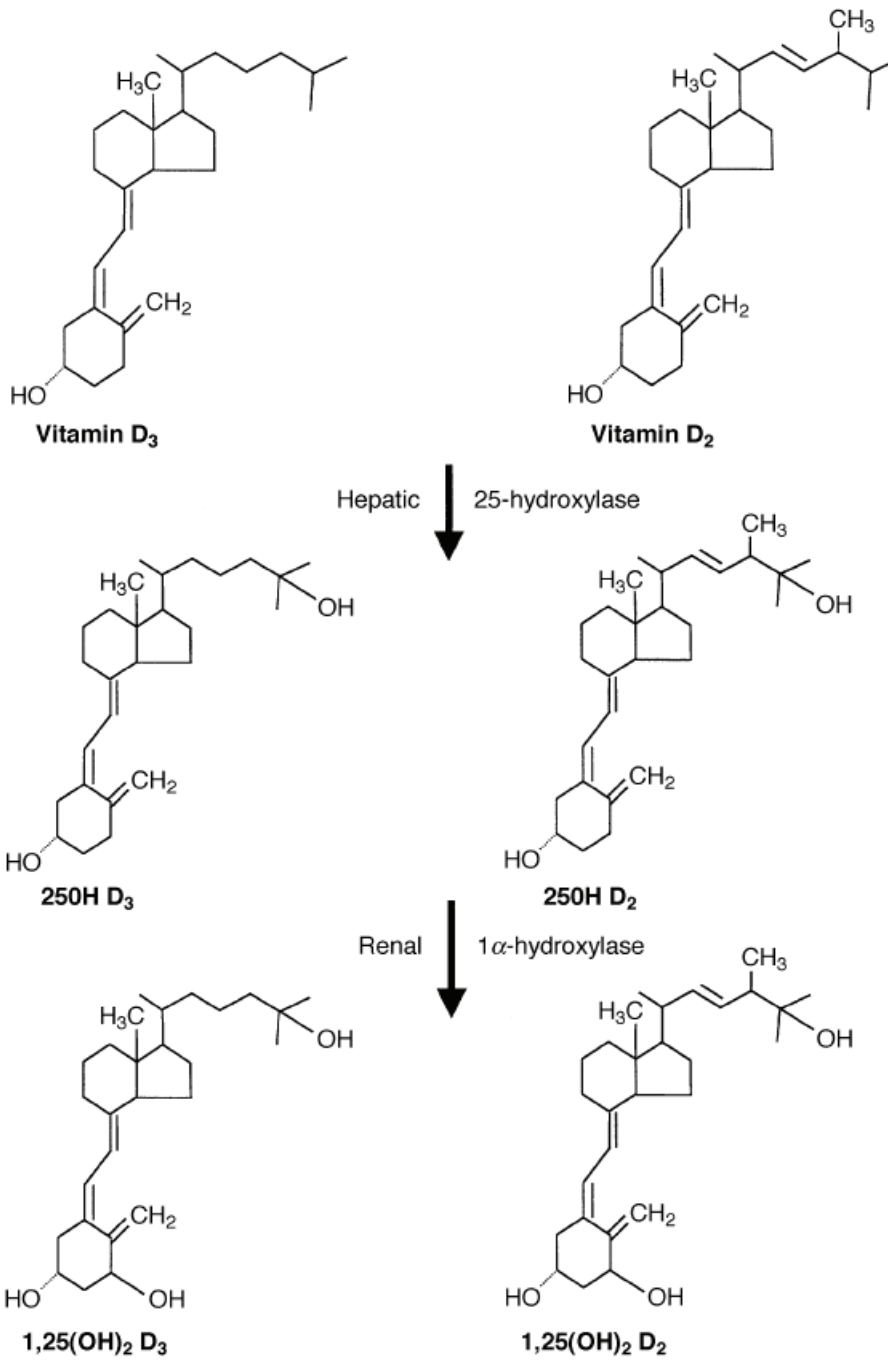
2.4 Vitamin D

Ever since vitamin D was discovered by Edward Mellamby in 1919 (Mellamby 1919) during his investigation into the causes of rickets, many new insights into its roles in disease prevention have emerged. Vitamin D is a family of compounds consisting of 9,10 secosteroids which differ in their side-chain structures (Mehta and Mehta 2002). Secosteroids have the same cyclopentanoperhydrophenanthrene ring structure as steroids but in secosteroids, two B-ring carbon atoms (C-9 and 10) out of the four steroid

rings are not joined giving it a “broken ring” appearance (Norman 2008, Deeb et al 2007).

There are five forms of vitamin D; vitamin D2, ergosterol; D3, cholecalciferol; D4, 22,23 dihydroergoalciferol; D5 sitosterol (24-ethylcholecalciferol) and D6 stigmasterol (Napoli et al 1979). The two main forms of vitamin D are vitamin D3, which is formed in the skin after exposure to sunlight or ultraviolet light and vitamin D2, which is obtained by irradiation of a few plant materials or food (DeLuca 2004, Holick and Garabedian 2006, Lips P 2006). Vitamin D does not naturally exist in significant quantities in the human food chain, because evolutionally humans have evolved a photosynthetic mechanism in their skin to produce large quantities of vitamin D3 (Hollis 2005).

Figure 5. Activation of vitamin D illustrating the structures of both forms of the vitamin: cholecalciferol (D3) and ergocalciferol (D2) Zerwekh 2004;41;272-281



2.4.1 Synthesis and metabolism

The generation of vitamin D3 starts in the skin after exposure to ultraviolet B (UVB) irradiation within the wavelength range of 290-315nm (Holick et al 1980). In animals, 7-dehydrocholesterol (7-DHC, also called pro-vitamin D) is synthesized de novo in the skin from Acetyl CoA via multiple steps (Glossmann 2010). Pro-vitamin D is distributed throughout the epidermis and dermis but is most abundant in the stratum spinosum and stratum basale.

When the skin is exposed to UVB within the range of 290-315nm, 7-DHC is photolytically converted to pre-vitamin D3 (Holick et al 1980). Pre-vitamin D3 is then non-enzymatically converted to vitamin D3 in a heat-dependent process i.e. the higher the temperature, the larger the amount of pre-vitamin D3 that isomerizes into vitamin D3 (WHO/IARC 2008). In order to regulate vitamin D3 production and prevent intoxication, pre-vitamin D3 is also converted to lumesterols, tachysterols and other inactive photoproducts such as suprasterols (Holick 1981, MacLaughlin 1982, Webb et al 1989). The conversion of pro-vitamin D to pre-vitamin D3 is very rapid, taking place in seconds while the isomerisation of pre-vitamin D3 to vitamin D3 can take hours (Tian et al 1993), hence circulating vitamin D concentrations are at their maximum level within 12-24 hours after UVB exposure (Adams et al 1982, Chen et al 2007).

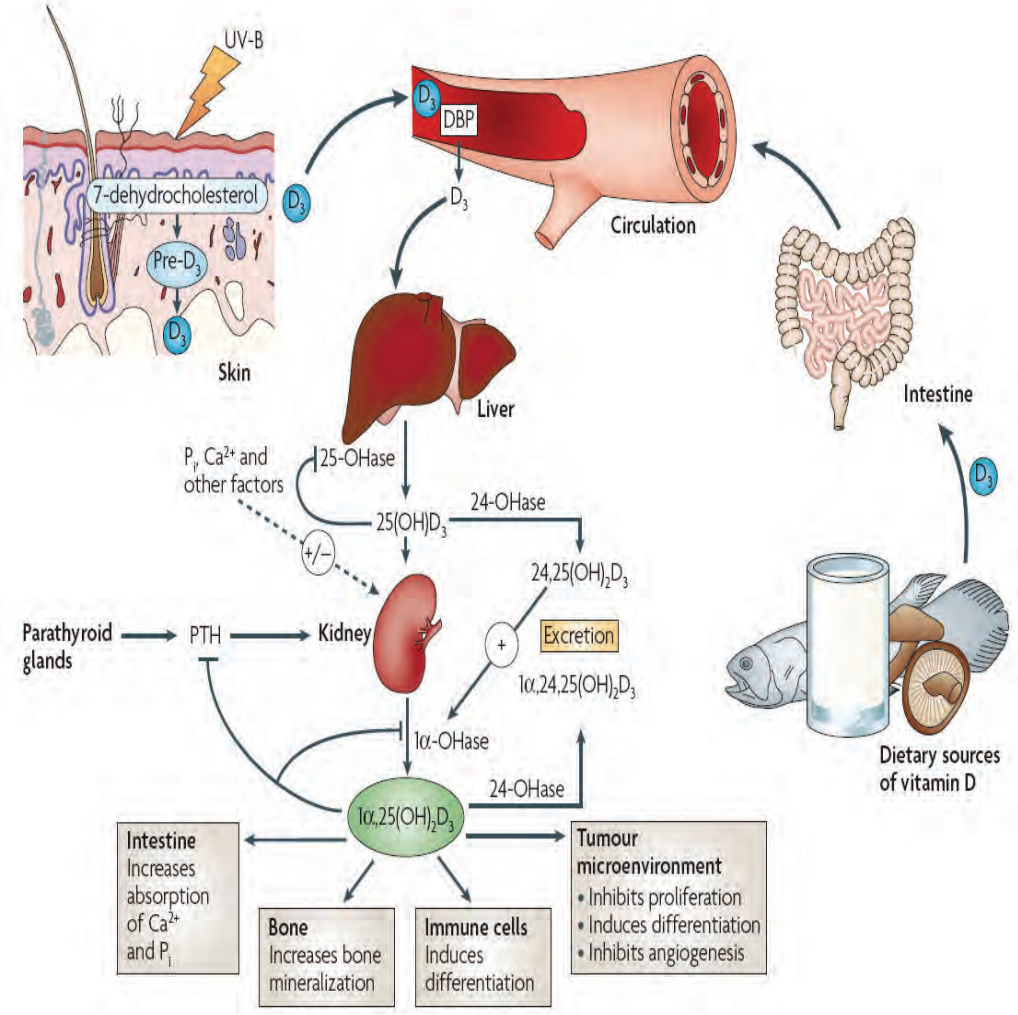
Vitamin D3 and vitamin D2 obtained from diet are subsequently incorporated into chylomicrons and transported by the lymphatic system into the venous circulation (Holick 2007). Within the circulation, vitamin D (vitamin D3 and vitamin D2 are in this context referred to jointly as vitamin D) is bound to vitamin D-binding protein (VDBP) which transports it to the liver where it is converted to 25-hydroxyvitamin D (25-OHD) by the 25-hydroxylase enzyme. This is the major circulating form of vitamin D; it has a long-half life, and can be used to determine vitamin D status (Holick 2007, WHO/IARC 2008). However, 25-OHD is inert by itself and needs to be converted to the hormone1α,25-dihydroxyvitamin D (1α,25-$(OH)_2$D) by 1α-hydroxylase and the candidate hormone, 24,25-dihydroxyvitamin D (24R,25-$(OH)_2$D3 by 24-hydroxylase enzyme in the kidney, under the action of parathyroid hormone, before it becomes biologically active (Fraser and Kodicek 1970, Dusso et al 2005, Norman 2008).

Compared to 25-OHD whose half-life is in weeks, that of 1α,25-$(OH)_2$D is in hours and its plasma concentrations are 1000-fold less than those of 25-OHD (Mullin and Dobbs 2007). Apart from its short half-life, 1α,25-$(OH)_2$D can not be used to determine vitamin D status because its serum levels will be normal or may even be elevated in deficiency states because of secondary hyperthyroidism (Hollick 2007). The 1α,25-$(OH)_2$D generated in the kidney is secreted into the circulation, bound to VBDP, and then

transported to target organs, where it induces genomic and non-genomic responses through its interaction with the vitamin D receptor (VDR) (Norman 2008, Prentice et al 2008).

The main target organs of 1α,25-$(OH)_2$D are the intestine, bone, parathyroid glands and the kidney itself, where it participates in the maintenance of calcium and phosphorus homeostasis. Initially, it was thought that hydroxylation of 25-OHD to 1α,25-$(OH)_2$D occurs only in the kidney but since 1981, it has been discovered that many extra-renal tissues posses the 1α,25-$(OH)_2$D enzyme and can produce 1α,25-$(OH)_2$D from 25-OHD (Barbour et al 1981, Bises et al 2004, Norman 2008). The locally produced 1α,25-$(OH)_2$D generates biological responses in these tissues, does not spill into circulation and therefore has very little effect on plasma 1α,25-$(OH)_2$D concentrations (Norman 2008, Prentice 2008). The regulation of 1α-hydroxylase at extra-renal sites is different from that of the kidney enzyme. At extra-renal sites, the regulation is mainly under the control of local factors such as cytokines and growth factors, which act to optimize local levels of 1,25-$(OH)_2$D (Dusso et al 2005).

Figure 6. Vitamin D metabolism. Reprinted from Deeb KK, Trump DL and Johnson CS. Vitamin D signalling pathways in cancer: potential for anticancer therapeutics Nature Rev Cancer 2007 with permission from Nature publishing group



2.4.2 Determinants of vitamin D production

Cutaneous synthesis of vitamin D within the UVB range of 290-315 nm accounts for most of an individual's nutritional requirements (Holick 2006). Thus, factors that affect cutaneous synthesis play important roles in maintaining vitamin D equilibrium and vitamin D status of an individual. These factors can be extrinsic and intrinsic

(i) Season; ultraviolet radiation is a function of its solar zenith angles (SZA) which is the angle between the local vertical and the position of the sun at any given time (Webb 2006, Kimlin 2008). All year long, the SZA changes because of the earth's rotation around the sun. During summer, the SZA's are small implying that the sun is more overhead and able to deliver more ultraviolet rays compared to winter when the SZAs are very large and oblique resulting in lower ultraviolet rays (Webb AR 2006, Kimlin 2008). Also, a UVB irradiation threshold of 18 mJ/cm_2 is required to induce vitamin D production (Matsuoka 1989), a level not reached during winter in northern areas (Hollis 2005).

(ii) latitude; at latitudes below 35^o, the solar zenith angle is very direct and cutaneous vitamin D production can occur all year long but at latitudes above 35^o, the SZA is very oblique during the winter months so that almost all the UVB photons below 315 nm are absorbed by the ozone layer preventing any cutaneous vitamin D production [Hollick 2004]. The higher the altitude, the longer the period when vitamin D can not be produced in the skin during winter (Kimlin 2008). Likewise, at lower altitudes, UVB travels through less atmosphere and particles and is thus more effective in generating vitamin D production.

(iii) Time of the day; UVB is mainly present in sunlight between 10am and 3pm (Holick 2004, Kimlin 2008). (iv) Air pollution, particulate matter within the atmosphere will reduce UVB levels while snow and sand reflect UVB (Webb 2006, Kimlin 2008).

(v) Skin pigmentation; melanin pigment competes for and absorbs UVB photons responsible for the photolysis of 7-DHC to pre-vitamin D3 (Clemens et al 1982). While this is a positive adaptive mechanism for dark skinned people living in tropical countries, it becomes a problem when they live in northern countries as they become liable to vitamin D deficiency. Also, the conversion rate of 7-DHC to pre-vitamin D3 is much slower in dark skinned people compared to light skinned people. While a light skinned individual will produce maximal pre-vitamin D3 with 10 minutes of exposure in a strong summer sun, a dark skinned individual will require almost 1 hour. A dark skinned person requires 5-10 times longer exposure to sunlight to produce

the same amount of vitamin D3 as a light skinned person (Clemens et al 1982).

Other factors include (vi) Age; elderly people are less exposed to sunshine, have less vitamin D3 precursors in their skin due to age-related changes in skin composition and the decline in renal function associated with ageing may also contribute (Holick et al 1989, Slovik et al 1981). (vii) Obesity, which is associated with low serum vitamin D levels and this is thought to be due to excessive storage of vitamin D metabolites in fat tissues leading to non-release into circulation (Parikh et al 2004, Wortsman et al 2000, Hypponen and Power 2007). (vii) Gender; women generally have lower serum vitamin D levels because of their higher amount of body fat (van Dam RM, Hypponen and Power 2007), moreover, people with (viii) fat malabsorption are prone to having low vitamin D levels (Haderslev 2003, WHO/IARC 2008).

2.4.3 Rate limiting steps in production

Because vitamin D is toxic at high doses, its production is in a state of continuous equilibrium in order to optimise plasma concentrations. In light skinned individuals, 7-DHC preferentially converts to inactive isomers, lumisterol and tachysterol, rather than pre-vitamin D3 after more than ten minutes exposure to UVB but as soon as pre-vitamin D3 stores are depleted, exposure of lumisterol and tachysterol to UVB encourages photoisomerisation of these isomers back to pre-vitamin D3 (Holick 1981, MacLaughlin 1982). This implies that less than 15% of all 7-DHC undergoing photoisomerisation at any single point in time will be converted to pre-vitamin D3 (WHO/IARC 2008). Likewise, after adequate amounts of vitamin D3 have been formed, continuous exposure of the skin to sunlight causes its rapid degradation into many inactive products such as transvitamin D, suprasterols 1 and suprasterols 2 (Webb et al 1989). According to calculations made in Boston, USA, during summer, 1 and 3 hrs of exposure to sunlight resulted in 75% and 95% vitamin D3 photodegradation respectively. Thus prolonged exposure to sunlight does not cause linear increases in vitamin D production (Webb et al, 1989).

Within the circulation, the most important limiting factor is the regulation of 1α,25-$(OH)_2$D production (Norman 2008). Serum calcium, phosphorus, fibroblast growth factor 23 (FGF-23) can either upregulate or downregulate 1α,25-$(OH)_2$D production depending on need. Likewise, 1α,25-$(OH)_2$D can decrease its own synthesis through negative feedback by decreasing the synthesis and secretion of PTH form the pituitary gland and by increasing the expression of 24-hydroxylase (24-OHase) enzyme (Dusso et al 2004, Henry 2005, Holick 2007, Deeb et al 2007). The 24-OHase enzyme catalyzes a series of oxidation reactions at carbons 24 and 23 leading to a

side chain cleavage and inactivation to the biologically inactive calcitroic acid (Dusso et al 2004, Deeb et 2007). Mice lacking a functional 24-hydroxylase gene have high serum $1,25(OH)_2D$ levels due to the decreased capacity to degrade it (St.-Arnaud et al 1996). Likewise, 24-OHase is regulated in a reciprocal manner by 1α-OHase (Dusso et al 2004).

2.4.4 Vitamin D from diet

Vitamin D is not a vitamin in the true sense because it is formed in the body. Very few food substances contain vitamin D. The best dietary sources of vitamin D3 are fresh oily fish. Abundant vitamin D3 sources include wild salmon and cod-liver oil, fresh, farmed or canned salmon, sardines, mackerel, herring and tuna fish (Bouillon 2001, Holick 2007, Lips 2006, Chen et al 2007). The major sources of vitamin D2 are mushrooms (e.g. Shiitake, Oyster, chanterelles etc). When Shiitake mushroom is sun-dried, the vitamin D2 content is 15 times more than the fresh type (Hollick 2007). In many western countries, milk, yoghurts, butter, margarine, cheese, cereals, orange juice and infant formulas are fortified with vitamin D3 but these are very small, usually 100IU/8 oz compared to the 600-1000IU/3.5 oz present in fresh, wild salmon (Hollick 2007).

2.4.5 Concepts of vitamin D deficiency, insufficiency and sufficiency

Serum 25-OHD concentrations are used in determining vitamin D status. Previously, vitamin D deficiency was defined as serum 25-OHD level less than 25 nmol/L because clinical evidence of skeletal diseases (rickets or osteomalacia) become manifest below this level (Wolpowitz and Gilchrest 2006). Because of emerging information on the myriad of vitamin D actions, definition of vitamin D deficiency has extended beyond its relationship with skeletal health alone. In vitamin D literature, the term deficiency is gradually being replaced by insufficiency. Vitamin D insufficiency is defined as the serum vitamin D level associated with adverse health outcomes within the population. This corresponds to a serum 25-OHD level of ≤ 50 nmol/L (Malabanan et al 1998, Hollick 2006, Bischoff-Ferrari et al 2006).

Physiologically, the threshold for vitamin D sufficiency is the maximum serum 25-OHD level beyond which no association exists between further increases in serum 25-OHD level and further decreases in serum PTH (Wolpowitz and Gilchrest 2006). In essence, this refers to the 25-OHD level needed for maximal suppression of PTH, which has been variously estimated to be between 70 and 80 nmol/L (Thomas et al 1998, Chapuy et al 1997, Heaney et al 2003). An expert consensus, however, has adopted a serum 25-OHD level of 75 nmol/L to indicate vitamin D sufficiency (Dawson-

Hughes et al 2005, Bischoff-Ferrari et al 2006). Serum 25-OHD levels between 52 and 72 nmol/L are considered as relative insufficiency (Holick 2007). Since intake of 1 mcg/day of vitamin D increases the serum 25-OHD concentrations by about 1 nmol/L (Heaney et al 2003), it is estimated that a daily vitamin D intake between 800 IU and 1500 IU (20 mcg and 37.5 mcg) is necessary to attain and maintain sufficient serum vitamin D concentrations but the requirements will be higher in people with circumstances which already predispose to vitamin D deficiency (Hollick 2007, Vieth 2004). In perspective, in many countries, 1liter of milk contains only 100 IU of vitamin D which will only increase plasma 25-OHD concentrations by between 2-3 nmol/L.

2.4.6. Vitamin D Receptor

Vitamin D receptor (VDR) is a nuclear receptor that modulates the biological activities of the active form of vitamin D. The VDR gene is located on chromosome 12q12q14p and is made up of promoter and regulatory regions and exons, spanning 75kb, which encode domains of the full length VDR protein (Haussler et al 1998, WHO/IARC 2008).

The VDR protein is made up of 3 regions; the NH2-terminal region containing a ligand-independent transactivation function, the central region contains the DNA binding domain, which targets the VDRE receptors, and the C-terminal region, which contains the ligand binding domain and RXR heterodimerization motif (Deeb et al 2007). The ligand binding domain is responsible for the high-affinity binding of $1,25(OH)_2D$. After ligand binding, conformational changes occur in the VDR structure (Dusso et al 2004).

The vitamin D-VDR complex heterodimerizes with retinoid X receptors (RXR), but before it can become active, it needs to be translocated to the nucleus, where it modulates transcription by binding to specific DNA elements in the promoter regions of the vitamin D response elements (VDRE) (Cheskis and Freedman 1994, Prufer et al 2000). Co-activators such as steroid receptor coactivator (SRC) and Creb binding protein 300 (CBP300), which modify chromatin enzymatic activities or histone acetylase activities play important roles in VDR mediated transcription (Wang 2009). VDR was initially thought to be involved only in calcaemic regulation, but recent studies have discovered that it is also involved in regulating cell proliferation, differentiation and immunodulation. It is ubiquitous and present in many tissues and cells within the body, but at different concentrations (Nagpal et al 2005, Bouillon et al 2006).

The VDR can modulate the expression of VDREs in three ways (Nagpal et al 2005): (i) by positively regulating the expression of specific genes by binding to the VDREs in their promoter regions (Sutton and MacDonald 2003,

Pinette et al 2003), (ii) by negatively regulating the expression of other genes by binding to negative VDREs (Liu et al 1996, Dong et al 2003) (iii) inhibiting gene expression by antagonizing the activities of some transcription factors (Alroy et al 1995, Nagpal et al 2005). It is estimated that the VDR directly regulates the expression of about 200 genes which include osteocalcin, osteopontin, carbonic anhydrase II, calbidin, interleukins 2 and 12, tumour necrosis factor-a, p21, p27 (Lomri and Baron 1992, Quelo 1998; D'Ambrosio et al 1998, Tobler 1987, Nagpal et al 2005) and may indirectly regulate another 300 genes (Carlberg 2003).

Many polymorphisms of the VDR gene occur with considerable differences between races but the most commonly encountered polymorphisms, *FokI, BsmI, ApaI and TaqI* are found in the intron separating exon VIII and IX (Morrison et al 1994, Dusso et al 2004). The polymorphisms may be synonymous (*Bsml, Apal, Taql and Tru9l*) and non-synonymous (*Fokl*) (Deeb et al 2007). The functional effects of these polymorphisms are not very clear but e.g. when *Fokl* polymorphism occurs at translation initiation, the result is a smaller VDR with greater transcriptional activity than the full length VDR (Deeb et al 2007).

2.4.7 Mechanisms of action of vitamin D

2.4.7.1 Genomic actions

Vitamin D generates biological responses by regulating gene expression after binding to the VDR (Deeb et al 2007). The VDR is a steroid hormone receptor which regulates gene expression in a ligand-dependent manner (Evans 1988). Formation of the ligand-receptor complex results in conformational changes in the receptor protein, which allows the complex to interact with specificity with other proteins that participate in the transcription process (Norman 2008). The anti-proliferative effects of vitamin D are mediated through the genomic pathways (Ylikomi et al 2002). Genomic actions of vitamin D take place over a long period of time; maybe even days (Wang 2009).

2.4.7.2 Non-genomic actions

Non-genomic actions mediated by vitamin D are very rapid, usually occurring in minutes and do not require transcription. They are mediated through the initiation of many signal transduction systems including calcium influx, calcium release from intracellular stores, modulation of adenylate cyclase, phospholipase C, protein C kinase pathways (Lehmann and Meurer 2010). The VDR and 1α,25-$(OH)_2$D-membrane-associated rapid response

steroid binding protein (1α,25-$(OH)_2$D-MARRS) modulate the non-genomic actions of vitamin D (Dusso et al 2004). The major non-genomic action of vitamin D is the rapid intestinal absorption of calcium brought about when vit D-VDR complex activates signalling cascades such as protein kinase C (PKC) resulting in rapid opening of cellular voltage-gated calcium channels ensuring an increase in intracellular calcium (Deeb et al 2007, Dusso et al 2004).

2.4.8 Clinical functions of vitamin D

The classical function of vitamin D is to maintain adequate serum calcium levels and thus, prevent rickets in children and osteomalacia and osteoporosis in adults (DeLuca 2004). It does this in three different ways
(i) it stimulates the active absorption of calcium, together with phosphate in the intestine. It is the only hormone known to induce the proteins necessary for active calcium absorption in the intestine (DeLuca 2004),
(ii) when intestinal calcium absorption is low or absent due to dietary deficiencies, vitamin D mobilizes calcium from skeletal sources by stimulating osteoblasts to produce receptor activator nuclear factor-κB ligand (RANKL) which in turn induces pre-osteoclasts to become mature osteoclasts (Suda et al 2002). Mature osteoclasts then cause bone resorption by removing calcium and phosphate from bones and making them available within the circulation,
(iii) in the kidneys, vitamin D acts synergistically with PTH to increase the reabsorption of calcium from the distal tubules, thereby preventing its excretion in urine.

Aside its classical functions, there is increasing evidence that vitamin D may be involved in the prevention of some cancers (prostate, breast, colorectal and ovaries). The protective effect of vitamin D on breast cancer risk has been observed among pre-menopausal and post-menopausal women (Abbas et al 2009, Abass et al 2010). While a large study observed that vitamin D is important in preventing prostate cancer progression (Li et al 2007), others have not noted any relationship between serum vitamin D levels and prostate cancer risk (Travis et al 2009, Park et al 2010). An important similarity in the biology of ovarian, breast and prostate cancer is that the three cancers are sex-steroid hormone responsive which underlies the possibility of an effect by vitamin D. Vitamin D has also been observed to have protective effects in autoimmune diseases such as type 1 diabetes, multiple sclerosis, Crohn's disease, systemic lupus erythematosus, psoriasis, and hypertension (Holick 2008, Nagpal et al 2005). It is plausible for vitamin D to have this wide range of functions because virtually all tissues and many immune cells have a VDR.

Likewise, many cells and tissues express the 1α-hydroxylase enzyme, which implies that local conversion of 25-OHD to 1α,25-$(OH)_2$D, the active form, can take place in such tissues (Holick 2008).

2.4.9. Molecular actions of vitamin D in cancer

1. ***Antiproliferative effects:*** Progression through the cell cycle is driven by a family of protein kinases mainly the cyclin dependent kinases (CDK) and conversely, CDK inhibitors (CDKI) negatively regulate cell cycle progression by binding to and suppressing CDK activities (Hunter and Pines 1994, Sherr and Roberts 1995). Vitamin D modulates the cyclin pathways by regulating the expression of proteins p21 and p27. This results in inhibition of CDK, which inhibits cell proliferation by inducing G1 cell cycle arrest and withdrawal from cell cycle (Deeb et al 2007, Ingraham et al 2008). This is a cellular surveillance mechanism ensuring that if problems with DNA replication or repair occur, a cell cycle arrest will take place rather than forming aberrant DNA (Deeb et al 2007, Ingraham et al 2008).

   Vitamin D also regulates the activities of c-myc protooncogene which is a cell-cycle related protein that enhances CDK activity through functional inactivation of the CDK-inhibitors (Mitchell and El-Deiry 1999, Perez-Roger et al 1999). Some indirect effects of vitamin D on cell cycle regulation such as the upregulation of insulin growth factor binding protein 3 (IGFBP3), transforming growth factor- β (TGF- β) and downregulation of epidermal growth factor receptor (EGFR) signalling pathways also contribute to its antiproliferative effects (Yanagisawa et al 1999, Huynh et al 1998, Tong et al 1999).

2. ***Apoptosis:*** Apoptosis is programmed cell death, and disruptions in apoptotic pathways whereby damaged cells keep proliferating, accumulating mutations and evading destructions are major hallmarks of cancer cells (Ingraham et al 2008). Mutations to the p53 gene which discontinues cell cycle when there is DNA damage is found in more than half of all cancers (Ingraham et al 2008). Vitamin D regulates key mediators of apoptosis by inducing the expression of pro-apoptotic proteins such as Bax, Bad and Bak and suppressing the expression of anti-apoptotic proteins such as Bcl2 and Bcl-x (Ylikomi et al 2002, Deeb et al 2007). It can also induce apoptosis indirectly by increasing intracellular calcium levels thereby activating calcium-dependent pro-apoptotic caspase 12 and micocalpain (Mathiasen et al 2002). Various apoptotic mechanisms may however be involved in different cancer cells as seen in ovarian cancer cells, where vitamin D also induces apoptosis by down-regulating telomerase activity.

3. ***Angiogenesis:*** Loss of contact inhibition is seen early in many cancers (Muehlemann et al 2005). The cell-adhesion molecule, E-cadherin is essential for maintaining a polar conformation in epithelial cells and its activity can be regulated by vitamin D (Gniadecki et al 1997, Palmer et al 2001, Ingraham et al 2008). The E-cadherin gene is a tumour suppressor gene commonly absent in transformed cells and an indication of poor prognosis in colon cancer cells (Palmer et al 2001). Experimentally, high concentrations of vitamin D and its analogs decrease the invasiveness, inhibit angiogenesis and metastases of different cancer cell lines**.** In cultured malignant cells, vitamin D analogs can also down-regulate cell-invasion associated matrix metalloproteinases 2, 9 and serine proteinases (Mantell et al 2000, Koli and Keski-Oja 2000).

2.4.10 Vitamin D and ovarian cancer

2.4.10.1 Ecological studies

The first reports of a possible association between vitamin D and ovarian cancer were reported in ecological studies using sunlight as a proxy for vitamin D status. The studies observed higher ovarian cancer mortality rates among women living at higher altitudes compared to those living at lower altitudes (Decarli and La Vecchia 1986, Leftkowitz and Garland 1994). Leftkowitz and Garland reported that ovarian cancer mortality was inversely proportional to annual intensity of sunlight, and therefore suggested that sunlight may be protective against death from ovarian cancer. More recent studies in different parts of the world have, however, yielded varying results. While a worldwide study and two studies conducted in the USA observed inverse correlations between UVB and ovarian cancer mortality (Freedman et al 2002, Grant 2006, Grant and Garland 2006), one from Spain and another from Japan did not observe such inverse correlation (Mizoue 2004, Grant 2007). However, using ovarian cancer mortality rather than incidence may have inherent bias and not really reveal the association between sunlight and ovarian cancer because of arbitrary and iatrogenic differences in cancer survival in different geographical areas. Moreover, using mortality, rather than incidence does not distinguish between the effects of sunlight/UVB on preventing ovarian cancer and improving survival from ovarian cancer (Porojnicu et al 2007).

Nevertheless, ecological studies of ovarian cancer incidence and sunlight have also been contradictory. A study making use of data from 175 countries observed that the age-adjusted ovarian cancer incidence rates were highest in countries located at higher altitudes (Garland et al 2006). The study also noted that while UVB irradiance was inversely associated with ovarian

cancer, stratospheric ozone which reduces UVB transmission was positively associated with ovarian cancer. However, two other multinational studies did not observe any inverse relationship between ovarian cancer incidence and sunlight (Waltz and Chodick 2008, Tuohimaa et al 2007) despite the fact that one of them observed an inverse association between sunlight and some other cancers (Tuohimaa et al 2007).

2.4.10.2 Dietary studies

Dietary studies of the association between vitamin D and ovarian cancer have not yielded consistent results. The dietary studies have used food frequency questionnaires to determine the vitamin D content of different food substances. In a case-control study in Mexico, Salazar-Martinez and colleagues reported a 57% lower risk of ovarian cancer among women whose diet contained the highest amount of vitamin D compared to those with the lowest (Salazar-Martinez et al 2002). Even though the risk reduction was smaller in a similar study in Italy, women whose diet contained the highest amount of vitamin D still had a 30% reduced risk of ovarian cancer compared to those whose diet contained the lowest amounts (Bidoli et al 2001). On the contrary, four studies conducted in the USA did not observe lower risks of ovarian cancer among women who consumed food substances high in vitamin D relative to those who consumed less (Kushi et al 1999, Goodman et al 2002, Cramer et al 2001, Koralek et al 2006).

Thus from available ecological and dietary studies, no definite inferences can be drawn regarding the relationship between vitamin D and ovarian cancer.

2.4.10.3 Experimental studies

In vitro studies have shown that 1α,25-$(OH)_2$D can suppress ovarian cancer cell growth via various molecular mechanisms.

GADD45 is a p53, BRCA1 regulated nuclear protein that plays a role in cell cycle progression, maintenance of genomic stability and DNA repair (Smith 1996, Jin et al 2000). Jiang and colleagues demonstrated that 1α,25-$(OH)_2$D causes cell cycle arrest at the G_2/M transition by inducing GADD45 in ovarian cancer cells (Jiang 2003). Cell cycle arrest caused by 1α,25-$(OH)_2$D induction of GADD45 is dependent on time, dose and VDR but not on p53.

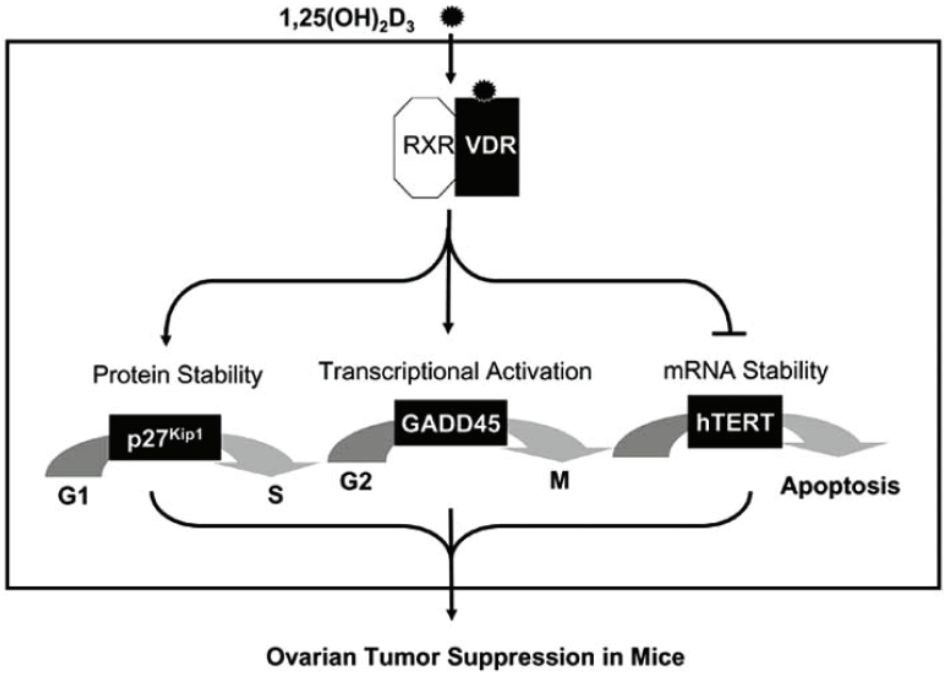
Li P et al also demonstrated that 1α,25-$(OH)_2$D arrests ovarian cancer cells in G_1 phase by increasing the accumulation and ensuring stability of the p27 protein, another known tumour suppressor (Li et al 2004). It achieves this by

(i) decreasing the amounts of cyclin E and cyclin E-associated CDK2 activity, ultimately resulting in less p27 phosphorylation and (ii) reducing the affinity of p27 for Skp2 ubiquitin ligase, the enzyme that causes its degradation (Zhang et al 2006).

In normal somatic cells, chromosomal ends (telomeres) undergo progressive shortening resulting in irreversible growth arrest but in cancer cells, the telomere length is stabilized by the enzyme telomerase (Bodnar et al 1998). Abnormally high levels of human telomerase reverse transcriptase (hTERT) are expressed in most cancer cells, including ovarian cancers. Vitamin D (1α,25-$(OH)_2$D) has been shown to down-regulate hTERT activity in ovarian cancer cells (Jiang et al 2004), thereby promoting their capability to induce growth arrest.

The study by Miettinen and colleagues offered new insights into the effects of vitamin D in ovarian cancer cells (Miettinen et al 2004). Their study revealed that high concentrations of 1α,25-$(OH)_2$D (10-100nM) were needed to inhibit ovarian cancer cell lines (OVCAR-3 cell lines) and low concentrations actually stimulated cell proliferation, similar to results obtained with prostate and breast cancer cell lines (Gross et al 1997, Love-Schimenti et al 1996). The vitamin D analogue, EB1089 (secocalcitol) displayed very strong growth inhibition of ovarian cancer cell lines at much lower concentrations compared to 1α,25-$(OH)_2$D. Another study has confirmed that experimentally, EB1089 suppresses ovarian cancer cell growth and activates the GADD45 gene much more effectively than 1α,25-$(OH)_2$D and in concentrations that do not induce hypercalcaemia (Zhang et al 2005).

Figure 7. Summary of the current understanding of the molecular mechanisms of action of vitamin D in ovarian cancer cells. Reprinted from Zhang X, Nicosia SV, Bai W. Vitamin D Receptor is a Novel Drug Target for Ovarian Cancer Treatment. Current Cancer Drug Targets 2006 with permission from Bentham Science publishers.



3 AIMS OF THE THESIS

The major aim of this thesis is to elucidate the relationship between vitamin D and ovarian cancer using serial, pre-diagnostic serum vitamin D measurements
Specific objectives are

1. To determine the effect of storage time and sampling season on the stability of serum 25-hydroxyvitamin D in serum samples that have been stored at -25^{o}C for many years.

2. To investigate the association between serum 25-hydroxyvitamin D and ovarian cancer risk and to explore the impact of season of blood collection on the risk estimates.

3. To evaluate the independent and joint effects of serum 25-hydroxyvitamin D and serum calcium on ovarian cancer risk.

4. To ascertain whether long term vitamin D status can predict ovarian cancer risk.

4 MATERIALS AND METHODS

4.1 Finnish Maternity Cohort (FMC)

The Finnish Maternity Cohort (FMC) was established in 1983 by the National Institute for Health and Welfare, THL (formerly National Public Health Institute). After an informed consent has been obtained (since 2001), blood samples are withdrawn from pregnant women at the municipal maternity centres between the 8th and 13th weeks of gestation in order to screen for intra uterine infections caused by Treponema pallidum, human immunodeficiency virus and hepatitis B virus (Koskela et al 2000, Pukkala et al 2007). The informed consent covers the use of the left-over serum samples for epidemiological studies and according to the law on KTL, samples obtained before 2001 can also be used for such studies. The samples are mailed from the municipal maternity centres to the THL's prenatal serology laboratory in Oulu. After routine screening, the left over sera are then stored in polypropylene cryo vials at -25 ºC in a well protected biorepository in Oulu. The average bench lag time (time between sampling and storage) is 4 days (range 1 to 10 days). Other personal data collected and registered in the FMC database include personal identity code, parity and number of gestations, date of sample collection at both municipal maternity centres and at prenatal serology laboratory in Oulu, date of sample processing, place or residence at birth and at sample collection.

More than 98% of pregnant women in Finland have donated serum samples to the FMC since inception and the biorepository presently contains about 1.6 million serum samples. Approximately 60,000 new serum samples are added to the biorepository every year.

4.2 Finnish Cancer Registry (FCR)

The FCR is nationwide. Cancer registration began in Finland in 1952 and since 1953 when the first complete nationwide cancer registration was conducted; over 950,000 cancer cases have been registered at the FCR (Pukkala 2001). Compulsory notification of all diagnosed or suspected cancer cases by physicians, laboratories and hospitals commenced in 1961 (Pukkala 2001, www.cancer.fi). The coverage of the FCR is virtually complete with no loss to follow-up and for solid tumours; the registration is over 99% complete (Teppo et al 1994). The ICD-O-3 coding nomenclature which takes into consideration the tumour site, morphology, behaviour and

grade has been used by the FCR since 2005. Coding is done by qualified secretaries under the supervision of the Registry physician.

Linkage to other registries and database such as the FMC, Statistics Finland is done through the use of a unique 11-digit personal identity code mandatory to every resident of Finland since 1961. The personal identity code is indicated on cancer registry forms. The cancer registry file containing personal identity codes is annually matched through computerized linkage with the cause of death register at the Statistics Finland so that dates and cause of deaths in cancer patients can be added to the FCR records (Pukkala 2001). It is also regularly linked with the Central Population Register to ensure that the personal identity codes are correct. The FMC was linked to the FCR to obtain the cases and controls used in this thesis.

Standards are rigorously maintained to ensure that there is no breach of confidentiality and individual data are well protected. In order to use data from the FCR for linkage and research purposes, approval needs to be obtained from the relevant ethical research committee, THL and other pertinent (e.g. data protection) authorities (Pukkala 2001).

4.3 Study population

Study I

Only first and second pregnancy samples were used for this study. Randomly, four women who donated serum samples during their first pregnancies were selected every other year between 1984 and 2002 such that two women donated serum samples during winter and the other two, during summer. The women were matched for age and municipality of residence. For this study, we defined winter as 15th December to 15th of March while summer was defined as 1st of May to 31st of August. In all, there were 201 summer samples and 201 winter samples available for pick up at the FMC biorepository. Furthermore, among the selected women, 100 who had donated blood samples to the FMC at their second pregnancies, provided the second pregnancy occurred within five years of the first, were identified. From this 2nd group, 40 women who donated their second samples within the same season and another 40 who donated their second samples during the opposite season were selected.

Study II

Two hundred and one ovarian cancer cases diagnosed within ten years of serum sampling were randomly selected from the FMC. If a case had donated more than 1 serum sample during this 10-year period, the sample donated closest to the time of cancer diagnosis was selected. The median

time between serum sampling and cancer diagnosis was 5 years. In most cases, the histology of the tumour was serous (39%) or mucinous (35.9%) ovarian carcinomas.

We matched two different sets of controls that were alive and free of cancer at the diagnosis of the index case to each case. While the two sets of controls were equally matched to cases for age at sample withdrawal ±1 year and parity, the first set of controls (2 per case, 398 serum samples available) were matched for date of blood sampling ±4 weeks (same season, most ideal practice) and the second set of controls were matched for opposite season of index blood sampling (1 per case approximately 6 months apart ±4 weeks, 199 samples available).

Studies III and IV

After linkage to the FCR, we identified ovarian cancer cases who had donated pre-diagnostic serum samples to the FMC on more than one occasion, at least one year before cancer diagnosis. We excluded cases who had participated in the second study. Thus, we had 172 cases, whose histological diagnoses were serous (41%), mucinous (36%) or endometroid (9%) ovarian carcinoma.

Controls were women from the FMC who were alive and free of cancer at the time of diagnosis of the index case and who had been pregnant/donated serum samples on at least two separate occasions. One control per case was matched for age at sample withdrawal ± 1 year, (ii) parity, and (iii) date of index blood sampling ± 2 weeks for both sets of samples. In essence, we had two sets of samples for each case-control pair. A control had to fulfil the matching criteria for both sampling periods.

For the 4th study, only cases and controls who donated their paired serum samples during the same season were selected leaving 53 case-control pairs whose 1st and 2nd samples were donated during winter and 37 case-control pairs whose 1st and 2nd samples were donated during summer.

4.4 Laboratory methods

All samples were coded before they were sent to the laboratories in order to ensure subject anonymity. Laboratory personnel were blinded to case-control status. Samples from cases and their matched controls were arranged sequentially in a random order, analysed with the same batches of assay kits and on the same day to minimise assay drift bias.

Study I

For each subject, one set 160 µl aliquots of each sera was sent to the departments of Anatomy, University of Tampere, Finland, for 25-OHD measurement, and another set was sent to Clinical Chemistry Laboratory, University of Umeå, Umeå, Sweden for androstenedione measurement. Quantification of 25-OHD was performed using 25-OHD IDS radioimmunoassay (IDS Ltd, Boldon UK). The interassay coefficients of variation (CV) were 4.0% and 3.9% at 25-OHD concentrations of 26.5 nmol/L and 103nmol/L respectively. Androstenedione concentrations were measured by competitive chemilumiscent enzyme immunoassay (DPC Immulite 2000 Androstenedione Chemilumiscent EIA, UK). The interassay coefficients of variation were 16.9% at 2.2 ng/ml and 6.2% at 5.2 ng/ml.

Studies II, III and IV

Measurement of 25-OHD was performed at the Department of Medical Biosciences, University of Umeå, Umeå, Sweden using a 25-OHD radioimmunoassay (IDS Ltd, Boldon UK). The within, between, and total coefficients of variations (CV) of the assay were 7.8%, 9.6% and 12.4% at level 28.4 nmol/L 25-OHD, and 4.1%, 7.4% and 8.5% at 107 nmol/L 25-OHD, respectively. The manufacturer stated a specificity of 100% for 25-OHD3, 75% for 25-OHD2, 100% for 24, 25-OH2D3, and less than 0.01% or 0.3% cross-reactivity for cholecalciferol (D3), and ergocalciferol (D2), respectively. Quantification of serum calcium was performed at the Clinical Chemistry Laboratory, Östersunds Hospital, Östersund, Sweden, using the Roche/Hitachi Cobas C system analyzer (Roche Diagnostics GmbH, D68298 Mannheim, Germany).

4.5 Statistical methods

All statistical analyses were carried out using SPSS for windows (SPSS Inc., Chicago IL). Versions 14 and 15 were used for the first two studies, while version 18 was used for the last two studies. In all studies, two-sided $p < 0.05$ was considered statistically significant. Descriptive statistics was calculated for all the variables in each study; presented as means (standard deviation or range) for normally distributed data and median (percentiles) for skewed data. Where appropriate, data were log-transformed to reduce departure from normal distribution. Correlations were assessed with Pearson correlation coefficient. In studies II, III and IV, conditional logistic regression was used to calculate the relative risk of ovarian cancer (expressed as odds ratio with 95% confidence interval, OR, 95% CI) across the different levels of serum 25-OHD concentrations.

Study I

Clustered box plots, stratified for sampling season were drawn to depict the relationship between the two hormones and duration of sample storage. We used Pearson's correlation coefficient to test for the correlation between duration of sample storage and 25-OHD and androstenedione concentrations. The effects of sampling season on single and paired hormone measurements were assessed using independent and paired sample t-tests respectively.

Study II

Quintile cut-off points were determined using the 25-OHD distribution among the controls, separately for controls with the same season sampling (as cases) and for controls with different sampling season (as cases). The multivariate model was adjusted for age at last full term pregnancy and bench lag time. Secondary analyses were conducted for the main histological groups available in the study (serous and mucinous tumours) and comparing women with sufficient serum 25-OHD (> 75 nmol/L) concentrations to those without. Tests for trend were performed using serum 25-OHD as a continuous variable.

Study III

Quartile cut-off points for both 25-OHD and calcium were determined using the distribution among the controls. Conditional logistic regression was used to calculate odds ratio with 95% confidence interval (OR with 95% CI) for ovarian cancer in the different quartiles of 25-OHD and calcium using the lowest quartiles as the reference category. Tests for trend were calculated using continuous scale of the variables, log-transformed for 25-OHD because the overall distribution was slightly skewed even though the season specific distributions were normal. The multivariate model was adjusted for age at first full term pregnancy and region of residence because they were the only variables that affected the risk estimates by more than 5%. Secondary analyses were conducted among cases whose lag times to cancer diagnosis were more than 3 years and their matched controls and among women with sufficient levels of serum 25-OHD compared to those with insufficient levels.

To determine the independent and joint effects of the two biomarkers on ovarian cancer risk, the women were categorized into four groups based on their combined serum 25-OHD and calcium levels (i) Low 25-OHD /low calcium - women with both 25-OHD and calcium categories within the 1st 3 quartiles, i.e. (25-OHD Q1, Q2, Q3 + Calcium Q1, Q2, Q3). This represented the reference category, (ii) high 25-OHD/low calcium – women whose 25-OHD were within the 4th quartile but whose calcium were within the 1st 3 quartiles (25-OHD Q4 + calcium Q1, Q2, Q3), (iii) low 25-OHD/high calcium – women with 25-OHD within the 1st 3 quartiles but calcium within the 4th

quartile (25-OHD Q1, Q2, Q3 +calcium Q4), (iv) high 25-OHD/high calcium - both 25-OHD and calcium were within the 4th quartiles (25-OHD Q4 + calcium Q4). We tested whether calcium modifies the effect of vitamin D in ovarian cancer and vice versa. This testing was done with a likelihood ratio test to compare two nested models, by considering the difference between the model-specific scaled deviances.

Study IV

Four groups were formed depending on the median 25-OHD concentrations for both 1st and 2nd samples. (i) Women whose 25-OHD concentrations were below the season-specific median values during both 1st and 2nd sampling periods, (ii) women with 25-OHD concentration below the median value during the 1st sampling period but above the median value during the 2nd sampling periods, (iii) women with 25-OHD concentration above the median value during the 1st sampling period but below the median value during the 2nd sampling period, (iv) women whose 25-OHD concentrations were above the median values during the 1st and 2nd sampling periods. The first group comprising women with 25-OHD levels below median values during both 1st and 2nd sampling period was used as the reference category. The model was adjusted for age at first full term pregnancy and region of residence. Women who donated their paired samples during winter and those who donated their paired samples during summer were analyzed separately.

5 RESULTS

Study I

Effect of sampling season and storage time on serum 25-OHD and androstenedione concentrations

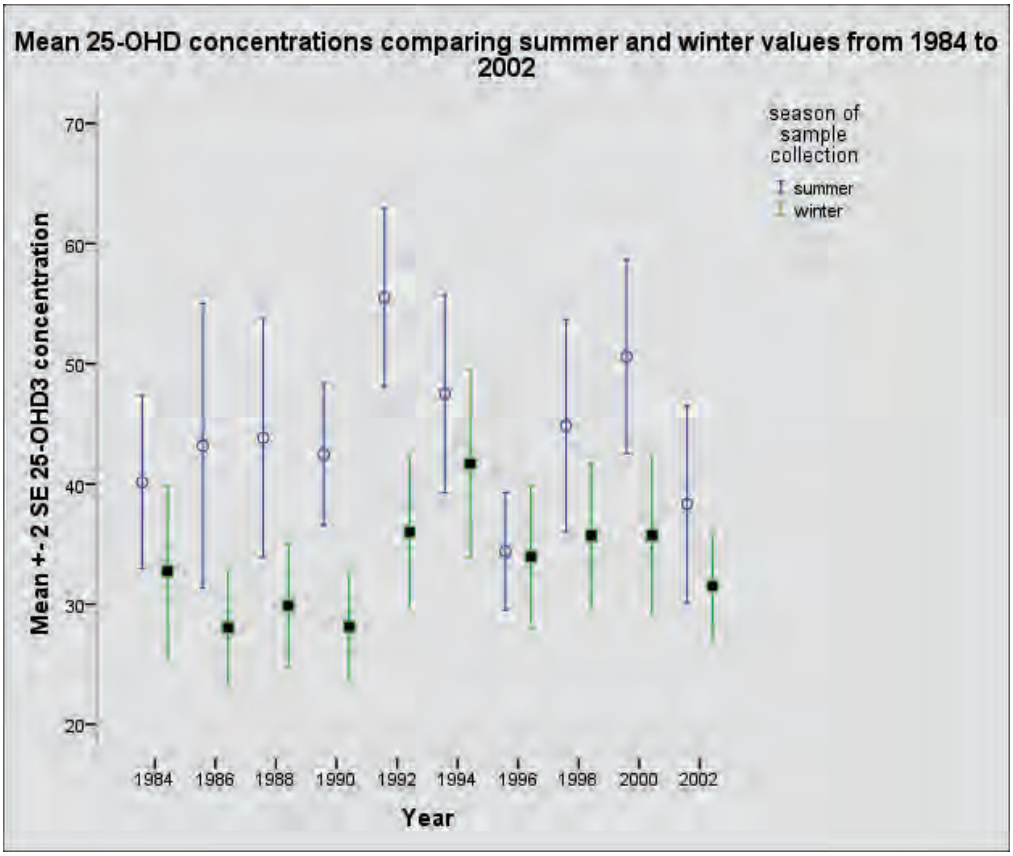
There was no correlation between serum 25-OHD concentrations and storage time for both first (r_s = -0.08, p-value = 0.1) and second pregnancy samples (r_s = -0.09, p-value = 0.5). The results did not differ by season of blood donation. The correlations between 25-OHD concentrations and storage time for samples donated in summer were 0.00 (p-value 0.98) and -0.11 (p-value 0.59) for first and second pregnancy samples respectively while for winter samples, they were -0.08 (p-value 0.64) and -0.09 (p-value 0.59) for first and second pregnancy samples respectively.

Similarly, there was no correlation between serum androstenedione concentrations and storage time (r_s = 0.09). Mean serum 25-OHD concentrations were highest in 1992 (44.8 nmol/L, 95% CI 39.2 - 50.5) and 1994 (44.5 nmol/L, 95% CI 38.8 - 50.2) and lowest in 1996 (34.2 nmol/L, 95% CI 30.4 – 38.3) and 2002 (34.7 nmol/L 95% CI 30.1 – 39.3). No corresponding differences were observed for the serum androstenedione levels.

Mean serum 25-OHD concentrations were significantly higher during summer compared to winter for both the first (44 nmol/L vs 33.4 nmol/L, p ≤0.001) and the second (51.6 nmol/L vs 39.8 nmol/L, p ≤0.001) pregnancy samples over all the years investigated. Conversely, androstenedione levels appeared to be higher in winter compared to summer but the difference in the mean levels was of borderline significance, mean difference μ_d= 0.3 nmol/ml, 95% CI 0.0-0.6; p-value = 0.05).

Among women who donated two samples, individual serum 25-OHD concentrations were significantly higher during summer compared to winter irrespective of pregnancy order. Also the mean 25-OHD concentrations for winter-winter sample combinations were significantly lower compared to other sample combinations such as summer-summer and summer-winter combinations. No corresponding differences were observed for androstenedione.

Figure 8. Mean 25-hydrovitamin D concentrations (± 2 standard errors) by season of blood donation from every two years from1984 to 2002



Study II

Association between serum 25-OHD concentrations and ovarian cancer risk

The median lag time between serum sampling and cancer diagnosis was 5 years. Among cases and controls that were tightly matched season of blood donation, low serum 25-OHD concentration was associated with an increased risk of ovarian cancer. Women with insufficient serum 25-OHD concentrations (< 75 nmol/L) were at borderline increased risk of ovarian cancer compared to women with sufficient serum concentrations, OR 2.7, 95% CI 1.0-7.9). The odds ratio, OR, comparing women within the lowest to highest quintile of serum 25-OHD concentration was 1.8, (95% CI 0.9-3.5, p-trend = 0.26). Among women who were matched for opposite season of

blood donation (6 months ±4 weeks), no differences in risk estimates were observed comparing the extreme quintiles: odds ratio 1.1 (95% CI 0.6-2.2, p-trend = 0.49). Analyses were carried out among the two most prevalent histological types of ovarian cancer in the study (serous and mucinous). Comparing lowest to highest quintiles, the OR for serous tumours was 1.4 (95% CI 0.7-3.2) and 1.5 (95% CI 0.4-3.1) for mucinous tumours

Table 1. Relative risks (Odds ratio, OR, with 95% confidence interval, CI) of ovarian cancer by quintile of serum vitamin D concentration in fertile aged Finnish women followed up for up to 10 years after serum withdrawal

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	P_{trend}
Among cases and controls who donated serum in same season (± 4 weeks)						
Quintile values, nmol/L	< 26.4	26.5–32.8	32.9–39.8	39.9–53.1	≥ 53.2	
n, cases, control	46/79	41/81	41/81	44/78	28/79	
OR, crude	1.6(0.9–2.9)	1.4(0.8–2.6)	1.4(0.8–2.5)	1.6(0.9–2.7)	1.0(reference)	
OR, adjusted[1]	1.8(0.9–3.5)	1.6(0.8–3.1)	1.5(0.8–2.8)	1.6(0.9–2.9)	1.0 (reference)	0.26
Serous tumours						
n, cases, control	20/32	12/33	17/28	14/27	13/29	
OR, adjusted[1]	1.4(0.6-3.6)	0.7(0.3-1.9)	1.6(0.6-4.4)	1.0(0.4-2.6)	1.0(reference)	
Mucinous tumours						
n, cases, control	15/26	17/29	14/26	15/32	9/24	0.49
OR, adjusted[1]	1.5(0.5-4.7)	1.6(0.6-4.7)	1.4(0.5-3.8)	1.3(0.5-3.5)	1.0(reference)	

[1]Adjusted for age at last full term pregnancy

Study III

Relationship between serum 25-OHD, calcium concentrations and the risk of ovarian cancer

There was no correlation between serum 25-OHD and serum calcium concentrations among cases and controls. Even though the season matched mean serum 25-OHD levels were higher among controls compared to cases for all the seasons, the differences in the mean levels were not statistically significant. The highest difference in the mean levels was observed during spring (8.5nmol/L).

Adjusting for age at first pregnancy and region of residence, women within the highest fourth of serum 25-OHD concentrations tended to have reduced risk of ovarian cancer compared to women within the lowest fourth but the trend test was not statistically significant (OR 0.57 95% CI 0.26–1.24; P-trend = 0.07). The odds ratio reduced further and the trend test became statistically significant when cases with lag time less than 3 years were excluded from the analysis (OR 0.43, 95% CI 0.18-1.05, P-trend = 0.02). Women with sufficient 25-OHD concentrations (>75 nmol/l) had a significantly reduced risk of ovarian cancer compared to those with insufficient serum concentrations (OR 0.32; 95% CI 0.12-0.91, p-value 0.03).

Women within the highest quartile of serum calcium had significantly reduced risk of ovarian cancer compared to those within the lowest quartile; OR 0.41 (95% CI 0.19–0.85, P-trend = 0.004). Exclusion of cases with lag time less than 3 years had no material effect on the point estimate; OR 0.37 (95% CI 0.16-0.85, P-trend 0.002).

We sought evidence of multiplicative interaction between calcium and vitamin D. Compared to women who had low calcium/low 25-OHD concentrations, those who had high calcium/high 25-OHD levels had an odds ratio of 0.26 (95% CI 0.07-0.90). We, however, observed no evidence to suggest that calcium modifies the effect of vitamin D (p = 0.25) or vitamin D modifies the effect of calcium (p = 0.12) on ovarian cancer risk

Table 2. Relative risk (estimated as odds ratio, OR, with 95% confidence intervals, CI) of ovarian cancer by quartile of serum 25-hydroxyvitamin D and calcium concentrations among Finnish women followed up to 13 years after sample donation

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P-trend	Q4 vs Q1-3
Serum 25-OHD concentrations						
Quartile values, nmol/L	<31.5	31.5–40.0	40.1–57.7	≥57.8		
Cases/control, n[1]	42/43	44/44	52/42	30/43		
OR, adjusted[2]	1.0 (reference)	1.01 (0.54–1.87)	1.13(0.60–2.12)	0.57(0.26–1.24)	0.07	0.54(0.28–1.02)
Excluding cases who donated serum samples within 1 to 3 years of cancer diagnosis						
Quartile values	<32.0	32.1-40.5	40.6-58.1	≥58.2		
Cases/control, n	36/33	37/33	35/35	25/36		
OR, adjusted[2]	1.0 (reference)	0.93 (0.48-1.82)	0.79 (0.39-1.63)	0.43 (0.18-1.05)	0.02	0.49 (0.24-1.01)
Serum calcium concentrations						
Quartile values,mmol/L	<2.2	2.2–2.4	2.4–2.6	≥2.6		
Cases/control, n[1]	46/39	50/45	48/44	26/44		
OR	1.0(reference)	0.99(0.54–1.85)	0.86(0.46–1.60)	0.46(0.23–0.95)	0.005	
OR, adjusted[2]	1.0(reference)	1.04(0.55–1.96)	0.84(0.44–1.61)	0.41(0.19–0.85)	0.004	
Excluding cases who donated serum samples within 1 to 3 years of cancer diagnosis						
Quartile values	<2.2	2.2-2.4	2.4-2.6			
Cases/control, n	35/31	43/35	37/32	20/39		
OR, adjusted[2]	1.0 (reference)	1.26 (0.59-2.67)	0.97 (0.46-2.08)	0.37 (0.16-0.85)	0.002	

[1]Controls were matched for age at serum sampling, parity and date of blood donation (± 2 weeks)
[2]Adjusted for age at first full term pregnancy and region of residence

Table 3. Joint effect of serum 25-hydroxyvitamin D and calcium on the relative risk (estimated as odds ratio, OR, with 95% confidence intervals, CI) of ovarian cancer among Finnish women

	Group 1	Group 2	Group 3	Group 4
Case/control, n	116/96	26/32	21/33	5/11
OR, adjusted[1]	1.0 (reference)	0.51 (0.29–1.05)	0.41(0.19–0.87)	0.26(0.07–0.90)
Excluding cases with lag times > 3 years				
Case/control, n	92/73	21/25	16/28	4/11
OR, adjusted[1]	1.0 (reference)	0.47 (0.20—1.09)	0.34 (0.14 -0.81)	0.19 (0.05 – 0.77)

Group 1- Low vitamin D/Low calcium
Group 2 - High vitamin D/Low calcium
Group 3 - Low vitamin D/High calcium
Group 4 - High vitamin D/High calcium
[1]Adjusted for age at first full term pregnancy and region of residence

Study IV

Long term vitamin D status and the risk of ovarian cancer risk in a longitudinal study

The average time difference between 1st and 2nd sample donation was 2.6 years. For controls, the correlation between 1st and 2nd sample serum 25-OHD concentrations was highly significant for summer combinations (rs = 0.60 (p-value ≤ 0.001) and moderately significant for winter combinations (rs = 0.39, p-value = 0.004). This was, however, not true for the cases: the correlation was 0.25 (p-value = 0.14) for summer combinations and 0.16 (p-value = 0.28) for winter combinations. For the matched summer pairs, controls had higher mean serum 25-OHD concentrations for both the first and second serum samples as compared to cases (55.4 vs 45.4 nmol/L for 1st samples and 57.4 vs 49.6 nmol/L for 2nd samples). This was not true for the matched winter pairs (37.7 vs 40.9 nmol/L for 1st samples and 37.8 vs 36.9 nmol/L for 2nd samples).

Among women who donated their two serum samples during summer season, having both serum 25-OHD concentrations above the median values compared to having serum 25-OHD levels below the median values was associated with an inverse risk of ovarian cancer, OR 0.21 (95% CI 0.05-0.99). A protective effect of increasing serum 25-OHD levels against ovarian cancer did, however, not reach statistical significance (p-trend = 0.06). Any over time effects were not observed among women who donated their two serum samples during winter.

Table 4. Table 2 Relative risk (estimated as odds ratio, OR, with 95% confidence intervals, CI) of ovarian cancer by long-term serum 25-0HD concentrations among women who donated at least two samples to the FMC between 1983 and 2006

	Group 1	Group 2	Group 3	Group 4	P-trend	Group4 vs others
Summer/Summer combination						
n cases/controls	18/15	5/11	4/4	13/4		
OR, adjusted[1]	1.0 (reference)	1.09(0.32-3.67)	0.56(0.11-2.88)	0.20(0.04-1.05)	0.06	0.21 (0.05-0.99)
Winter/Winter combination						
n cases/controls	16/17	10/13	10/10	16/15		
OR, adjusted[1]	1.0 (reference)	1.50(0.51-4.41)	1.24(0.42-3.66)	0.91(0.35-2.45)	0.93	0.81(0.33-1.95)

[1]Adjusted for age at first full term pregnancy and region of residence

6 DISCUSSION

In the following, we will discuss the limitations and strengths of our study setting and design, and how the new findings fit into the literature on the role of vitamin D in ovarian cancer

6.1 Use of biobank material and determination of serum 25-hydroxyvitamin D and androstenedione

Our observations that there was no correlation between the serum 25-OHD concentrations and the length of time the serum samples have been stored for is similar to other studies. Ocke et al (1994) had earlier observed very high correlation (rs = 0.81) between the serum 25-OHD concentrations of the same individual measured 4 years apart. This was recently confirmed by Jorde et al (2010) who reported similar correlation coefficient (rs = 0.80) between the serum 25-OHD concentrations of the same individual measured 1 year apart. The correlation coefficients for samples stored over a longer period of time, 14 years, however ranged between 0.42 and 0.52. Another recent study has observed that serum 25-OHD has excellent reproducibility with an intraclass correlation coefficient of 0.72 over a 3 year period (Kotsopoulos et al 2010). Bodnar et al used another method to determine the stability of serum 25-OHD in stored blood. They compared 25-OHD concentrations using two very important predictors of serum 25-OHD (season and race) in sera frozen for more that 40 years to those frozen for 2 years among pregnant women. Serum 25-OHD concentrations were significantly higher in summer samples compared to winter samples, and among white women compared to black women, irrespective of the time the samples were donated (Bodnar et al 2009) implying that long term storage had no deleterious effect on the validity of serum 25-OHD measurements in epidemiological studies.

Serum 25-OHD has also been noted to withstand multiple freeze-thaw cycles (Antoniucci et al 2004, Wielders and Wjnjberg 2008, Lewis and Elder 2008), and is not degraded under ambient room or outdoor temperature for up to 8 days as long as the sample is protected from direct sunlight (Lewis and Elder 2008). It is thought that the reason why vitamin D and its metabolites are very stable in serum/plasma is because they are completely bound to the vitamin D binding protein and the complex formed can withstand insults effectively (Hollis 2008).

The seasonal differences in serum 25-OHD concentrations we reported are one of the most consistent features of vitamin D metabolism and have been reported several times (Stamp and Round 1974, Lu et al 1992, Harris and

Dawson-Hughes 1998, Ono et al 2005). No previous studies had, however, explored the use of paired samples to investigate seasonal differences in serum 25-OHD concentrations. Our observation that paired samples taken during winter/winter had significantly lower serum 25-OHD levels compared to other seasonal combinations such as summer/summer and summer/winter is an indication that epidemiological studies making use of paired samples to investigate vitamin D status/disease associations must appropriately match the paired samples of cases and controls for seasonality.

There is no reason to believe that early pregnancy would have affected the serum 25-OHD concentrations and, by extension, measurements. It has been shown that early pregnancy serum 25-OHD concentrations remained constant and is within the pre-pregnancy range (Bruinse and van den Berg 1995). However, as pregnancy progresses longitudinally, there may be changes in serum 25-OHD levels which are mainly reflections of the different seasons a pregnancy traverses and the accompanying seasonal changes rather than inherent changes brought about by pregnancy. If exposure to sunlight and vitamin D intake are taken into consideration, 25-OHD concentrations in pregnancy reflect the non-pregnant state (Ardawi et al 1997). This was buttressed in our study where we observed no correlation between serum 25-OHD concentrations and number of days of gestation. Thus, first trimester serum 25-OHD level is representative of a woman's 25-OHD level.

Quantification of serum 25-OHD is the most clinically useful assessment of vitamin D status because its long half-life (3 weeks) provides an indication of vitamin D stores obtained from both UV radiation and long-term dietary intake. Likewise, the production of 25-OHD is primarily substrate dependent and there is very little regulation in the liver. Unlike 25-OHD, the physiologically active form of vitamin D, 1,25-OHD2, is not clinically useful in determining an individual's vitamin D status because it has a very short half-life (4-6 hours) coupled with the fact that its enzymatic conversion in the kidneys is very tightly regulated. In clinical practice, serum 1,25-OHD2 measurement is mainly used as a confirmatory test of some disorders of the vitamin D endocrine system such as pseudo-vitamin D deficiency rickets and hereditary 1,25-OHD2 resistant rickets (Zerwekh 2004).

Serum 25-OHD measurement encompasses both 25-hydroxycholecalciferol (25-OHD3) and 25-hydroxyergocalciferol (25-OHD2). Previously, both forms of 25-OHD used to be measured and reported separately because it was believed that this will distinguish the sunlight derived from the dietary derived form but since food is now fortified with both forms of 25-OHD and both forms are used in multivitamin supplements, the need to distinguish the two forms in measurement is no longer necessary. It is still important to note that total 25-OHD measured and reported in laboratories is made up mainly of

25-OHD3, with 25-OHD2 contributing only minimally, and as many as 99% of individuals in USA may have no circulating 25-OHD2 (Hollis 2008).

6.2 Serum 25-OHD concentrations and the risk of ovarian cancer

Our observation of a non-statistically significant increased risk of ovarian cancer among women with the lowest serum 25-OHD levels compared to those with the highest is similar to that reported by three other studies that have investigated this relationship (Tworoger et al 2007, Arslan et al 2009, Zheng et al 2010). Apart from the study by Arslan et al, the other 2 studies, however, observed that circulating 25-OHD appeared to be protective among certain subgroups: among overweight women (Tworoger et al 2007, Zheng et al 2010) and among women with sufficient plasma levels compared to those with insufficient plasma levels (Tworoger et al 2007). We also noted a borderline increased risk of ovarian cancer among women with insufficient serum 25-OHD concentrations compared to those with sufficient serum concentrations but we did not have the BMI data on this subset of cohort members to explore the effect of BMI on the relationship between 25-OHD and ovarian cancer risk. Overall, the results from our study support previous findings.

One similarity between all these studies is their prospective nature. Serum 25-OHD concentrations were determined in blood samples donated before cancer diagnosis. While two of the studies were nested case-control studies (Arslan et al 2009, Toriola et al 2009), the other two were pooled studies combining resources from 3 cohorts (Tworoger et al 2007) and 7 cohorts (Zheng et al 2010). The age at blood donation, however, differed markedly between pre-menopausal women in our cohort and the combined pre and post-menopausal women in the other studies, (median ages at blood donation; 31.5 years vs 51 to 58 years).

While all the studies appropriately matched controls to cases based on season, our study differs from other studies because, we further assessed the effects of improper matching for season on the results by matching another set of controls who donated serum samples at seasons different to the cases (6 months ± 4 weeks). We observed an attenuation of the relative risks in analysis carried out using these set of controls demonstrating the impact of proper seasonal matching in vitamin D and associated disease studies.

6.3 Independent and joint effects of calcium and 25-OHD on ovarian cancer risk

To the best of our knowledge, this study was the first to investigate an association and joint effects of serum calcium and 25-OHD with regard to ovarian cancer risk. Our observation of a strong inverse association between serum calcium and ovarian cancer is novel and intriguing. Previous studies on the relationship between calcium and ovarian cancer risk had employed the use of dietary calcium and have yielded conflicting results. Goodman et al (2002) reported significant inverse associations between both dietary and diary calcium intake and ovarian cancer risk but not with non-dairy calcium intake. Likewise, the use of calcium supplements appeared to be associated with a reduced risk of ovarian cancer. Bidoli and colleagues also reported a 30% significant risk reduction in ovarian cancer risk among women with the highest dietary calcium intake compared to those with the lowest (Bidoli et al 2001) while Koralek and colleagues observed borderline inverse associations between dietary calcium intake and ovarian cancer risk (Koralek et al 2006). Nevertheless, other studies, including a pooled analysis of cohort studies have not observed any protective effect for calcium on ovarian cancer risk, be it calcium from dietary or dairy sources (Kushi et al 1999, Genkinger et al 2006, Park Y et al 2009).

Serum calcium is the most ideal way to determine an individual's calcium levels and relating serum calcium, rather than dietary calcium to ovarian cancer risk represents the best way to determine the relationship between calcium and ovarian cancer. The reason is that despite the fact that diet is one of the major means of obtaining serum calcium, it has been observed that dietary calcium intake has no significant effect on serum calcium levels (Jorde et al 2001) because serum calcium level is a function of vitamin D, parathyroid hormone (PTH) levels, and other dietary factors (Nordin 2000, Jorde et al 2001, Steingrimsdottir 2005). In a diet containing 20 mmol of calcium, about 16 mmol are lost in faeces indicating that only 4 mmol are absorbed through the intestine (Ramasamy 2006).

Calcium may reduce ovarian cancer risk by mediating apoptosis, cell growth and proliferation (McConkey and Orrenius 1997, Ramasamy 2006) and down-regulating the production of PTH, a suspected tumour-promoter and mitogenic factor (McCarty 2000). The effects of vitamin D and calcium on disease processes may be synergistic with VDR and CaR signalling pathways converging on the same pathway (Peterlik et al 2009). The joint effect of vitamin D and calcium has been demonstrated with regards to colon, breast and some other cancers (Cho et al 2004; Sergeev 2004, Lappe et al 2007). A previous study had investigated the joint effects of calcium and lactose, but not calcium and vitamin D in ovarian cancer (Goodman et al 2002). The study noted an inverse association between calcium intake and

ovarian cancer which was modified by lactose intake wherein high calcium intake was beneficial among women with low lactose intake but not among women with high lactose intake.

Similar to other studies, (Tworoger et al 2007, Toriola et al 2009, Arslan et al 2009, Zheng et al 2010) we observed in our joint effect study a non-statistically significant reduction in ovarian cancer risk among women within the highest quartile of serum 25-OHD levels compared to those within the lowest quartile but in contrast to other studies, we noted that women with the highest serum 25-OHD levels had a borderline reduced risk of ovarian cancer compared to other all other women. Women with sufficient serum 25-OHD levels also had significantly reduced risk of ovarian cancer compared to women with insufficient levels; previous studies having reported borderline inverse associations (Tworoger et al 2007, Toriola et al 2009).

Both 25-OHD and calcium, however, acted independently and do not modify the effect of each other on ovarian cancer risk.

6.4 Long term vitamin D status and ovarian cancer risk

An acknowledged weakness in previous studies on the association between vitamin D status and ovarian cancer is the use of only one time serum 25-OHD concentrations in the determination of ovarian cancer risk. We attempted to overcome this in our study. In healthy individuals, serum 25-OHD concentrations do not change drastically over time (Jorde et al 2010, Hofmann et al 2010, Kotsopoulos 2010) except when measured in different seasons (Agborsangaya et al 2010). Therefore, long term vitamin D status as determined by at least 2 serum 25-OHD measurements taken from the same individual, years apart, is likely to reflect the individual's long term serum 25-OHD status and changes in the status.

In this study, cancer free women devoid of any cancer at the time when ovarian cancer diagnosis was made in the cases exhibited significant tracking in their serum 25-OHD concentrations during summer. A reduced risk of ovarian cancer was observed among women with consistently high serum 25-OHD concentrations during summer, suggesting deterioration of vitamin D status from continuously high/sufficient serum 25-OHD levels is a risk factor for the development of ovarian cancer. An experimental study has shown that while high concentrations of 1,25-OHD2 (100nM) inhibited growth of ovarian cancer cell lines, concentrations of 1nM had no effect on cell growth and 0.1nM stimulated cell growth underscoring the importance of always having high serum 25-OHD concentrations. Among those who donated their serum samples during winter, mean serum 25-OHD levels for cases and controls were very low which may have precluded our ability to observe any differences in risk.

6.5 Pathobiology of vitamin D, vitamin D receptor and ovarian cancer

Studies on polymorphisms in VDR gene and the risk of ovarian cancer also support the role of the vitamin D endocrine system in ovarian carcinogenesis. The VDR is present in both normal and malignant ovarian cells where it mediates the actions of vitamin D (Bouillon et al 1995, Ahonen et al 2000).

Lurie et al (2008) noted diverse patterns in ovarian cancer risk associated with VDR gene polymorphisms across different ethnic groups. Caucasian but not Japanese women who were heterozygous for the FokI f allele had increased risk of ovarian cancer compared with homozygous carriers. Likewise, while Caucasian women, who were heterozygous and homozygous ApaI A allele carriers were at increased risk of ovarian cancer compared with homozygous carriers of the ApaI a allele, Japanese women were not. The increased risk of ovarian cancer associated with the FokI genotype is functionally and biologically coherent because the f allele has an earlier start codon giving rise to a protein with three extra amino acids which has a lower transactivation of VDR target genes compared to the F allele (Jurutka et al 2000).

On the other hand, Japanese women who were heterozygotic carriers of the Cdx-2 A allele had reduced risks of ovarian cancer compared with the homozygotic G allele carriers. The Cdx-2 G→A SNP alters the binding site of CDX transcription factor, and the A allele binds more efficiently resulting in higher VDR activity (Yamamoto et al 1999, Uitterlinden et al 2004).

The above results have been replicated in a larger study whereby the FokI genotype was associated with increased risk of ovarian cancer and the Cdx-2 had no effect on risk among Caucasian women (Tworoger et al 2009).

Not only are VDR polymorphisms associated with risk of developing ovarian cancer, they are also associated with improved prognosis in patients with ovarian cancer (Tamez et al 2009). In stage I ovarian cancer, there was no difference in survival between women with C/C, C/T and T/T genotypes. But in stages II-IV cancers, 84% of women with C/C genotype were alive 30 months post surgery compared to 50% of patients with C/T and T/T genotypes (Tamez et al 2009), suggesting that the C/C genotype conferred some survival advantages compared to the others.

6.6 Limitations of the study

The following limitations of our study need to be taken into consideration. The use of oral contraceptives is a confounder of the relationship between serum 25-OHD concentrations and ovarian cancer risk because oral contraceptives increase serum 25-OHD concentrations (Harris and Dawson-Hughes 1998) and at the same time, protect against ovarian cancer. We did not have information on oral contraceptive use in our cohort but we presume the women would have been off OCP use for many weeks before the samples were taken, since the samples were taken between the 8th and 13th weeks of gestation. In other studies that have controlled for OCP use, including OCP in the model did not have any appreciable effects on the risk estimates. Hence, the possibility of oral contraceptive use being a confounder in this scenario may be slight but cannot be discarded.

A significant interaction has been observed between 25-OHD concentrations and BMI (Zheng et al 2010) and two studies have observed reduced risks of ovarian cancer among overweight women with high serum 25-OHD levels (Tworoger et al 2007, Zheng et al 2010) . We however did not have information on BMI in this subset of women within the FMC to investigate the effect of BMI on the relationship between serum 25-OHD levels and ovarian cancer risk.

Our study included only fertile, pre-menopausal women; hence we may not be able to generalize the results obtained from our study to infertile and post-menopausal women. Nevertheless, Pukkala and colleagues (Pukkala et al 2007) reported similar cancer incidence rates among women in the FMC compared to the general population, except for some tumours such as borderline tumours of the ovary, whose incidence rate is lower among women in the cohort compared to the general population.

In the 3rd study, we measured total calcium rather than ionized calcium. It is believed that ionized calcium, rather than total calcium may provide the best indication of an individual's calcium status because it is the biologically active form and is tightly regulated by calcium-regulating hormones (Almquist et al 2007). Total calcium levels depend to a large extent on serum albumin levels and conditions that may affect serum albumin levels may affect total calcium levels. A recent study on serum calcium and breast cancer however observed that adjusting for serum albumin levels did not substantially change the risk estimates (Almquist et al 2010). Barring any conditions that may affect serum albumin levels, total calcium is a good indicator of calcium homeostasis.

Our study was not large enough to accommodate extensive sub-group analysis. Likewise, the relatively small sample size precluded us from fully

investigating the relationship between serum 25-OHD concentrations and the various ovarian cancer histological subtypes since the relationship between serum 25-OHD concentrations and ovarian cancer will most likely differ by histological subtype because vitamin D is identical to the sex-steroid hormones and the hormonal risk factors for ovarian cancer differ according to histological subtype. This is especially true for the 3rd study where we could not investigate the possibility of calcium modifying the effect of vitamin D among the various histological subtypes and in the 4th study where we could not determine the risk of ovarian cancer among the various histological subgroups by long term serum 25-OHD concentrations.

6.7 Strengths of the study

Unlike ecological studies which related ovarian cancer risk to UVB exposure and other measures of sunlight, we determined the association between vitamin D and ovarian cancer using serum 25-OHD measurements in a longitudinal setting applying a prospective nested case-control design with serials samples. This is the best way to determine the individual's vitamin D status. The results from ecology studies are not adequate to determine the true nature of a relationship between vitamin D and ovarian cancer. Ecology studies are mainly good for deriving hypothesis but not for determining causalities. This is because ecological studies can only make inferences about populations since they do not reflect biological events at the individual level (Greenland S and Robins 1985). Furthermore, ecological studies have limited statistical prowess to measure the true relationship between exposure and outcome and confounding factors can not be entirely controlled (Waltz and Chodick 2008).

Our study design was prospective whereby the serum samples had been collected before cancer diagnosis thereby ensuring that the serum 25-OHD and calcium measurements are more likely to represent the pre-cancer state, rather than the peri or post-cancer state. The distinction between the use of pre-cancer and post-cancer serum samples to measure serum 25-OHD concentrations is very important since the effects of cancer on serum 25-OHD concentrations are not well known, and the possibility that ovarian cancer may have an affect on serum 25-OHD levels can not be ruled out. If the samples had been taken after cancer diagnosis, there would have been no way to determine if the associations observed were due to the effects of ovarian cancer on serum 25-OHD measurements, rather than vice versa. Furthermore, in the 2nd and 3rd studies, we conducted secondary analyses excluding women who donated serum samples within 2 to 3 years of cancer diagnosis in order to eliminate cases who may have had occult ovarian cancers and the results were not dissimilar to that obtained in the overall analysis. In addition, incident density sampling of cancer free controls and their serial samples enabled us to make inferences about the changes in

vitamin D status in relation to ovarian cancer risk. Overall, by making the study prospective in nature, the risk of inverse causation bias was greatly minimized.

The study was nested within the FMC ensuring that cases and controls were chosen from the same well-defined source population which greatly reduces the possibility of selection bias. Controls subjects were randomly selected, alive and free of ovarian cancer at the time of diagnosis of the index case and were tightly matched to cases on the basis of risk factors such as parity, age and season of blood donation all of which can distort the relationship between serum 25-OHD concentration and the risk of ovarian cancer if not properly controlled. The population-based nature of the FMC (encompassing 98% of all pregnant Finnish women, Pukkala E 2007) also offers fundamental strength to our study.

7 CONCLUSIONS

From this nested case-control study on vitamin D and ovarian cancer, we can draw the following conclusions

1. Serum samples, stored at -25oC in biobanks for upwards of 24 years, can be used to study vitamin D-disease associations because 25-OHD is stable in stored blood. Such studies must, however, match tightly for season of blood donation.

2. We found no overall association between serum 25-OHD concentrations and ovarian cancer risk but, we found evidence suggestive of an increased risk of ovarian cancer among women with insufficient serum 25-OHD concentrations compared to those with sufficient serum concentrations. Our results further underscored the need to tightly match for seasonality because there was evidence of bias in analysis involving samples not properly matched for season.

3. Vitamin D and calcium are independently protective against ovarian cancer. While increasing serum calcium concentrations were associated with a significantly reduced risk of ovarian cancer, increasing serum 25-OHD concentrations were associated with a borderline reduced risk of ovarian cancer. Women with sufficient serum 25-OHD concentrations had a significantly reduced risk of ovarian cancer compared to women with insufficient serum concentrations.

4. Over time vitamin D status may be the key to protection against ovarian cancer. In analysis carried out using two samples collected years apart from each woman, having consistently high serum 25-OHD levels during summer was associated with a decreased risk of ovarian cancer. No similar observations were, however, observed among women who donated serum samples during winter.

An important implication for future research is the need for a larger study using serial samples to determine the association between changes in vitamin D status and ovarian cancer risk.

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9 REFERENCES

Abbas S, Linseisen J, Slanger T, Kropp S, Mutschelknauss EJ, Flesch-Janys D, Chang-Claude J. Serum 25-hydroxyvitamin D and risk of post-menopausal breast cancer--results of a large case-control study. Carcinogenesis. 2008:29(1):93-9

Abbas S, Chang-Claude J, Linseisen J. Plasma 25-hydroxyvitamin D and premenopausal breast cancer risk in a German case-control study. Int J Cancer. 2009:124(1):250-5

Adami HO, Hsieh CC, Lambe M, Trichopoulos D, Leon D, Persson I, Ekbom A, Janson PO. Parity, age at first childbirth, and risk of ovarian cancer. Lancet 1994;344(8932):1250-4

Adams JS, Clemens TL, Parrish JA, Holick MF. Vitamin D synthesis and metabolism after ultraviolet irradiation of normal and vitamin D deficient subjects. N Eng J Med 1982;306:(12);722-5

Ahonen MH, Zhuang YH, Aine R, Ylikomi T, Tuohimaa P. Androgen receptor and vitamin D receptor in human ovarian cancer: growth stimulation and inhibition by ligands. Int J Cancer. 2000;86(1):40-6.

Almquist M, Bondeson AG, Bondeson L, Malm J, Manjer J. Serum levels of vitamin D, PTH and calcium and breast cancer risk-a prospective nested case-control study. Int J Cancer. 2010:1272159-68

Almquist M, Manjer J, Bondeson L, Bondeson AG. Serum calcium and breast cancer risk: results from a prospective cohort study of 7,847 women. Cancer Causes Control 2007:18(6):595-602

Alroy I, Towers TL, Freedman LP. Transcriptional repression of the interleukin-2 gene by vitamin D3: direct inhibition of NFATp/AP-1 complex formation by a nuclear hormone receptor.Mol Cell Biol 1995:15:5789–99

Antoniou AC, Gayther SA, Stratton JF, Ponder BA, Easton DF. Risk models for familial ovarian and breast cancer. Genet Epidemiol 2000:18(2):173-90

Antonuicci DM., Black DM and Sellmeyer DE: Serum 25-Hydroxyvitamin is unaffected by multiple freeze-thaw cycles. Clic Chem 2005: 51:258-61

Ardawi MS, Nasrat HA, BA'Aqueel HS. Calcium-regulating hormones and parathyroid hormone-related peptide in normal human pregnancy and postpartum: a longitudinal study. Eur J Endocrinol 1997:137(4):402-9

Arslan AA, Clendenen TV, Koenig KL et al. Circulating vitamin d and the risk of epithelial ovarian cancer. J Oncol 2009; doi:10.1155/2009/672492

Auersperg N, Edelson MI, Mok SC, Johnson SW, Hamilton TC. The biology of ovarian cancer. Semin Oncol 1998: 25:281–304

Barbour GL, Coburn JW, Slatopolsky E, Norman AW, Horst RL. Hypercalcemiain an anephric patient with sarcoidosis: evidence for extrarenal generation of 1,25-dihydroxyvitamin D. N Engl J Med 1981:305(8):440-3

Beral V; Million Women Study Collaborators, Bull D, Green J, Reeves G. Ovarian cancer and hormone replacement therapy in the Million Women Study. Lancet 2007:369(9574):1703-10

Bidoli E, La Vecchia C, Talamini R, Negri E, Parpinel M, Conti E, Montella M, Carbone MA, Franceschi S. Micronutrients and ovarian cancer: a case-control study in Italy. Ann Oncol 2001;12(11):1589-93.

Bischoff-Ferrari HA, Giovannucci E, Willett WC, Dietrich T, Dawson-Hughes B. Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. Am J Clin Nutr 2006:84(1):18-28

Bises G, Kállay E, Weiland T, Wrba F, Wenzl E, Bonner E, Kriwanek S, Obrist P, Cross HS. 25-hydroxyvitamin D3-1alpha-hydroxylase expression in normal and malignant human colon. J Histochem Cytochem 2004:52(7):985-9

Blaustein A, Kaganowicz A, Wells J. Tumor markers in inclusion cysts of the ovary. Cancer 1982: 49:722–6

Bodnar AG, Ouellette M, Frolkis M, Holt SE, Chiu CP, Morin GB, Harley CB, Shay JW, Lichtsteiner S, Wright WE. Extension of life-span by introduction of telomerase into

normal human cells. Science 1998:279(5349):349-52.
Bodnar LM, Catov JM, Wisner KL, Klebanoff MA: Racial and seasonal differences in 25-hydroxyvitamin D detected in maternal sera frozen for over 40 years. Br J Nutr 2008:23, 1-7
Bouillon R, Eelen G, Verlinden L, Mathieu C, Carmeliet G, Verstuyf A. Vitamin D and cancer. J Steroid Biochem Mol Biol 2006:102(1-5):156-62
Bouillon R, Okamura WH, Norman AW. Structure-function relationships in the vitamin D endocrine system. Endocr Rev 1995;16(2):200-57.
Bouillon R. Vitamin D: from photosynthesis, metabolism, and action to clinical applications. In: DeGroot LJ, Jameson JL, eds. Endocrinology. Philadelphia: W.B. Saunders, 2001:1009-28
Bray F, Loos AH, Tognazzo S, La Vecchia C. Ovarian cancer in Europe: Cross-sectional trends in incidence and mortality in 28 countries, 1953-2000. Int J Cancer 2005:113(6):977-90
Brinton LA, Gridley G, Persson I, Baron J, Bergqvist A. Cancer risk after a hospital discharge of endometriosis. Am J Obstet Gynecol 1997: 176; 572-9
Bruinse HW, van den Berg H. Changes of some vitamin levels during and after normal pregnancy. Eur J Obstet Gynecol Reprod Biol 1995:61(1):31-7
Carlberg C. Current understanding of the function of the nuclear vitamin D receptor in response to its natural and synthetic ligands. Recent Results Cancer Res 2003:164:29-42
Chapuy MC, Preziosi P, Maamer M, Arnaud S, Galan P, Hercberg S, Meunier PJ. Prevalence of vitamin D insufficiency in an adult normal population. Osteoporos Int 1997;7(5):439-43.
Chen TC, Chimeh F, Lu Z, Mathieu J, Person KS, Zhang A, Kohn N, Martinello S, Berkowitz R, Holick MF. Factors that influence the cutaneous synthesis and dietary sources of vitamin D. Arch Biochem Biophys 2007: 460(2):213-7
Chen VW, Ruiz B, Killeen JL, Coté TR, Wu XC, Correa CN. Pathology and classification of ovarian tumours. Cancer 2003;97(10 Suppl):2631-42.
Cheng W, Liu J, Yoshida H, Rosen D, Naora H. Lineage infidelity of epithelial ovarian cancers is controlled by HOX genes that specify regional identity in the reproductive tract. Nat Med 2005:11: 531–37.
Cheskis B, Freedman LP. Ligand modulates the conversion of DNA-bound vitamin D3 receptor (VDR) homodimers into VDRretinoid X receptor heterodimers. Mol Cell Biol 1994:14:3329–38
Cho E, Smith-Warner SA, Spiegelman D, Beeson WL, van den Brandt PA, Colditz GA, Folsom AR, Fraser GE, Freudenheim JL, Giovannucci E, Goldbohm RA, Graham S, Miller AB, Pietinen P, Potter JD, Rohan TE, Terry P, Toniolo P, Virtanen MJ, Willett WC, Wolk A, Wu K, Yaun SS, Zeleniuch-Jacquotte A, Hunter DJ. Dairy foods, calcium, and colorectal cancer: a pooled analysis of 10 cohort studies. J Natl Cancer Inst 2004 ;96(13):1015-22.
Clemens TL, Adams JS, Henderson SL, Holick MF. Increased skin pigment reduces the capacity of skin to synthesise vitamin D3. Lancet 1982:1(8263):74-6
Collaborative Group on Epidemiological Studies of Ovarian Cancer, Beral V, Doll R, Hermon C, Peto R, Reeves G. Ovarian cancer and oral contraceptives: collaborative reanalysis of data from 45 epidemiological studies including 23,257 women with ovarian cancer and 87,303 controls. Lancet 2008:371(9609):303-14
Cramer DW, Hutchison GB, Welch WR, Scully RE, Ryan KJ. Determinants of ovarian cancer risk. I. Reproductive experiences and family history. J Natl Cancer Inst 1983:71(4):711-6
Cramer DW, Kuper H, Harlow BL, Titus-Ernstoff L. Carotenoids, antioxidants and ovarian cancer risk in pre- and postmenopausal women. Int J Cancer 2001;94(1):128-34.
D'Ambrosio D, Cippitelli M, Cocciolo MG, Mazzeo D, Di Lucia P, Lang R, Sinigaglia F, Panina-Bordignon P. Inhibition of IL-12 production by 1,25-dihydroxyvitamin D3. J Clin Invest 1998;101:252–62
Danforth KN, Tworoger SS, Hecht JL, Rosner BA, Colditz GA, Hankinson SE, Breastfeeding and risk of ovarian cancer

in two prospective cohorts. Cancer Causes Control 2007:18:517-23
Dawson-Hughes B, Heaney RP, Holick MF, Lips P, Meunier PJ, Vieth R. Estimates of optimal vitamin D status. Osteoporos Int 2005;:16(7):713-6
Decarli A, La Vecchia C. Environmental factors and cancer mortality in Italy: correlational exercise. Oncology 1986:43(2):116-26.
Deeb KK, Trump DL, Johnson CS. Vitamin D signalling pathways in cancer: potential for anticancer therapeutics. Nat Rev Cancer 2007:7(9):684-700
Deligdisch L, Einstein A, Guera D, Gil J. Ovarian dysplasia in epithelial inclusion cysts. Cancer 1995: 76:1027–34
DeLuca HF. Overview of general physiologic features and functions of vitamin D. Am J Clin Nutr 2004:80:Suppl:1689S-96S.
DeLuca HF. Overview of general physiologic features and functions of vitamin D. Am J Clin Nutr 2004 :80(6 Suppl):1689S-96S
Domchek SM, Rebbeck TR. Prophylactic oophorectomy in women at increased cancer risk. Curr Opin Obstet Gynecol 2007:19(1):27-30
Dong X, Craig T, Xing N, Bachman LA, Paya CV, Weih F, McKean DJ, Kumar R, GriffinMD. Direct transcriptional regulation of RelB by 1_,25-dihydroxyvitamin D3 and its analogs: physiologic and therapeutic implications for dendritic cell function. J Biol Chem 2003: 278:49378–85
Dubeau L. The cell of origin of ovarian epithelial tumors and the ovarian surface epithelium dogma: does the emperor have no clothes? Gynecol Oncol 1999: 72: 437–42
Dubeau L. The cell of origin of ovarian epithelial tumours. Lancet Oncol 2008:9;1191-97
Dusso AS, Brown AJ, Slatoposky E. Vitamin D. AM J Physiol Renal Physiol 2005: 289;8-28
Evans RM. The steroid and thyroid hormone receptor superfamily. Science 1988:240;(4854);889-95
Fairfield KM, Hankinson SE, Rosner BA, Hunter DJ, Colditz GA, Willett WC. Risk of ovarian carcinoma and consumption of vitamins A, C, and E and specific carotenoids: a prospective analysis. Cancer. 2001:92(9):2318-26.
Ford D, Easton DF, Stratton M, Narod S, Goldgar D, Devilee P, Bishop DT, Weber B, Lenoir G, Chang-Claude J, Sobol H, Teare MD, Struewing J, Arason A, Scherneck S, Peto J, Rebbeck TR, Tonin P, Neuhausen S, Barkardottir R, Eyfjord J, Lynch H, Ponder BA, Gayther SA, Zelada-Hedman M, et al. Genetic heterogeneity and penetrance analysis of the BRCA1 and BRCA2 genes in breast cancer families. The Breast Cancer Linkage Consortium. Am J Hum Genet 1998: 62(3);676-89
Fraser DR and Kodicek E. Unique biosynthesis by kidney of a biological active vitamin D metabolite. Nature 1970:228: 764–6
Freedman DM, Dosemeci M, McGlynn K. Sunlight and mortality from breast, ovarian, colon, prostate, and non-melanoma skin cancer: a composite death certificate based case-control study. Occup Environ Med 2002: 59(4):257-62.
Garland CF, Mohr SB, Gorham ED, Grant WB, Garland FC. Role of ultraviolet B irradiance and vitamin D in prevention of ovarian cancer. Am J Prev Med 2006: 31(6):512-4.
Genkinger JM, Hunter DJ, Spiegelman D, Anderson KE, Arslan A, Beeson WL, Buring JE, Fraser GE, Freudenheim JL, Goldbohm RA, Hankinson SE, Jacobs DR Jr, Koushik A, Lacey JV Jr, Larsson SC, Leitzmann M, McCullough ML, Miller AB, Rodriguez C, Rohan TE, Schouten LJ, Shore R, Smit E, Wolk A, Zhang SM, Smith-Warner SA. Dairy products and ovarian cancer: a pooled analysis of 12 cohort studies. Cancer Epidemiol Biomarkers Prev 2006:15(2):364-72.
Gilks CB, Prat J. Ovarian cancer pathology and genetics: recent advances. Hum Pathol. 2009: 40(9):1213–23
Gilks CB, Prat J. Ovarian carcinoma pathology and genetics: recent advances. Hum Pathol 2009: 40(9):1213-23.
Gniadecki R, Gajkowska B, Hansen M. 1,25-dihydroxyvitamin D3 stimulates the assembly of adherens junctions in keratinocytes: involvement of protein kinase C. Endocrinology 1997:138(6):2241-8.
Goldberg JM, Piver MS, Jishi MF, Blumenson L. Age at onset of ovarian cancer in

women with a strong family history of ovarian cancer. Gynecol Oncol 1997: 66(1):3-9

Goodman MT, Wu AH, Tung KH, McDuffie K, Kolonel LN, Nomura AM, Terada K, Wilkens LR, Murphy S, Hankin JH. Association of dairy products, lactose, and calcium with the risk of ovarian cancer. Am J Epidemiol 2002:156(2):148-57.

Grant WB, Garland CF. The association of solar ultraviolet B (UVB) with reducing risk of cancer: multifactorial ecologic analysis of geographic variation in age-adjusted cancer mortality rates. Anticancer Res 2006: 26(4A):2687-99.

Grant WB. An ecologic study of cancer mortality rates in Spain with respect to indices of solar UVB irradiance and smoking. Int J Cancer 2007:120(5):1123-8.

Grant WB. The likely role of vitamin D from solar ultraviolet-B irradiance in increasing cancer survival. Anticancer Res 2006: 26(4A):2605-14.

Green A, Purdie D, Bain C, Siskind V, Russell P, Quinn M, et al. Tubal sterilisation, hysterectomy and decreased risk of ovarian cancer. Survey of women's health study group. Int J Cancer 1997:71;948-51

Green A, Purdie D, Bain C, Siskind V, Russell P, Quinn M, Ward B. Tubal sterilisation, hysterectomy and decreased risk of ovarian cancer. Survey of Women's Health Study Group. Int J Cancer 1997:71(6):948-51

Greenland S, Robins JM. Confounding and misclassification. Am J Epidemiol 1985:122(3):495-506.

Gross C, Peehl DM, Feldman D. Vitamin D and prostate cancer. In: Feldman D, Glorieux FH, Pike JW. Vitamin D. San Diego: Academic Press 1997; 1125-39

Grossmann HH. Origin of 7-Dehydrocholesterol (pro-vitamin D) in the skin. J Invest Dermatol 2010:130:(8);2139-41

Haderslev KV, Jeppesen PB, Sorensen HA, Mortensen PB, Staun M. Vitamin D status and measurements of markers of bone metabolism in patients with small intestinal resection. Gut 2003:52(5);653-8

Hankinson SE, Colditz GA, Hunter DJ, Spencer TL, Rosner B, Stampfer MJ. A quantitative assessment of oral contraceptive use and risk of ovarian cancer. Obstet Gynecol 1992:80(4):708-14

Hankinson SE, Hunter DJ, Colditz GA, Willett WC, Stampfer MJ, Rosner B, Hennekens CH, Speizer FE. Tubal ligation, hysterectomy, and risk of ovarian cancer. A prospective study. JAMA 1993:270(23):2813-8

Harris SS and Dawson-Hughes B. The association of oral contraceptive use with plasma 25-hydroxyvitamin D levels. J Am Coll Nutr 1998:17(3):282-4

Harris SS, Dawson-Hughes B. Seasonal changes in plasma 25-hydroxyvitamin D concentrations of young American black and white women. Am J Clin Nutr 1998:67(6):1232-6.

Haussler MR, Whitfield GK, Haussler CA, Hsieh JC, Thompson PD, Selznick SH, Dominguez CE, Jurutka PW. The nuclear vitamin D receptor: biological and molecular regulatory properties revealed. J Bone Miner Res 1998:13(3):325-49

Heaney RP, Davies KM, Chen TC, Holick MF, Barger-Lux MJ. Human serum 25-hydroxycholecalciferol response to extended oral dosing with cholecalciferol. Am J Clin Nutr 2003:77(1):204-10.

Heaney RP, Dowell MS, Hale CA, Bendich A. Calcium absorption varies within the reference range for serum 25-hydroxyvitamin D. J Am Coll Nutr 2003:22(2):142-6.

Henry HL. The 25-hydroxyvitamin D 1-hydroxylase. In: Feldman D, Pike JW, Glorieux FH, eds. Vitamin D. San Diego, CA: Elsevier Academic Press, 2005:69–83

Hofmann JN, Yu K, Horst RL, Hayes RB, Purdue MP. Long-term variation in serum 25-hydroxyvitamin D concentration among participants in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial.Cancer Epidemiol Biomarkers Prev 2010:19(4):927-31

Holick MF, MacLaughlin JA, Doppelt SH. Regulation of cutaneous previtamin D3 photosynthesis in man: skin pigment is not an essential regulator. Science 19 81: 211(4482): 590-3

Holick MF, Garabedian M. Vitamin D: photobiology, metabolism, mechanism of action, and clinical applications. In: Favus MJ, ed. Primer on the metabolic bone

diseases and disorders of mineral metabolism. 6th ed. Washington, DC: American Society for Bone and Mineral Research, 2006:129-37

Holick MF, MacLaughlin JA, Clark MB, Holick SA, Potts JT Jr, Anderson RR, Blank IH, Parrish JA, Elias P. Photosynthesis of previtamin D3 in human skin and the physiologic consequences. Science;1980: 210(4466);203-5

Holick MF, Matsuoka LY, Wortsman J. Age, vitamin D, and solar ultraviolet light. Lancet 1989: 2;1104–5

Holick MF. High prevalence of vitamin D inadequacy and implications for health. Mayo Clin Proc 2006:81:353–73

Holick MF. Vitamin D deficiency. N Eng J Med 2007:357;266-81

Holick MF. Vitamin D: a D-Lightful health perspective. Nutr Rev 2008:66(10 Suppl 2):S182-94

Holick MF. Vitamin D: importance in the prevention of cancers, type 1 diabetes, heart disease, and osteoporosis. Am J Clin Nutr 2004:79(3):362-71

Hollis BW. Circulating 25-hydroxyvitamin D levels indicative of vitamin D sufficiency: implications for establishing a new effective dietary intake recommendation for vitamin D. J Nutr 2005:135(2):317-22.

Hollis BW. Measuring 25-hydroxyvitamin D in a clinical environment: challenges and needs. Am J Clin Nutr 2008:88(suppl);507S-10S

Holschneider CH and Berek JS. Ovarian cancer: epidemiology, biology and prognostic factors. Seminars in Surgical Oncology 2000:19:3–10

Hunter T, Pines J. Cyclins and cancer. II: Cyclin D and CDK inhibitors come of age. Cell 1994: 79(4):573-82

Huynh H, Pollak M, Zhang JC. Regulation of insulin-like growth factor (IGF) II and IGF binding protein 3 autocrine loop in human PC-3 prostate cancer cells by vitamin D metabolite 1,25(OH)2D3 and its analog EB1089. Int J Oncol 1998:13(1):137-43.

Hypponen E, Power C. Hypovitaminosis D in British adults at age 45 y: nationwide cohort study of dietary and lifestyle predictors. Am J Clin Nutr 2007:85(3);860-8

Ingraham BA, Bragdon B, Nohe A. Molecular basis of the potential of vitamin D to prevent cancer. Curr Med Res Opin 2008:24(1):139-49.

International Agency for Research on Cancer, World Health Organization. Cancer incidence and mortality worldwide in 2008, GLOBOCAN 2008. Available online at file:///H:/Ovarian%20cancer/GLOBOCAN%202008/GLOBOCAN%202008.htm

Jaffe NS, ed. Pathology and Genetics: Tumours of haematopoietic and lymphoid tissues. Lyon: International Agency for Research in Cancer, 2003

Jiang F, Bao J, Li P, Nicosia SV, Bai W. Induction of ovarian cancer cell apoptosis by 1,25-dihydroxyvitamin D3 through the down-regulation of telomerase. J Biol Chem 2004 :279(51):53213-21

Jiang F, Li P, Fornace AJ Jr, Nicosia SV, Bai W. G2/M arrest by 1,25-dihydroxyvitamin D3 in ovarian cancer cells mediated through the induction of GADD45 via an exonic enhancer. J Biol Chem 2003:278(48):48030-40

Jin S, Zhao H, Fan F, Blanck P, Fan W, Colchagie AB, Fornace AJ Jr, Zhan Q. BRCA1 activation of the GADD45 promoter. Oncogene 2000:19(35):4050-7.

Jorde R, Sneve M, Hutchinson M, Emaus N, Figenschau Y, Grimnes G. Tracking of serum 25-hydroxyvitamin D levels during 14 years in a population-based study and during 12 months in an intervention study. Am J Epidemiol 2010: DOI: 10.1093/aje/kwq005

Jorde R, Sundsfjord J, Bønaa KH. Determinants of serum calcium in men and women: the Tromso study. Eur J Epidemiol 2001;:7(12):1117-23.

Jurutka PW, Remus LS, Whitfield GK, Thompson PD, Hsieh JC, Zitzer H, Tavakkoli P, Galligan MA, Dang HT, Haussler CA, Haussler MR. The polymorphic N terminus in human vitamin D receptor isoforms influences transcriptional activity by modulating interaction with transcription factor IIB. Mol Endocrinol 2000:14(3):401-20.

Kimlin MG. Geographic location and vitamin D synthesis. Mol Aspects Med 2008:29:453-61

King MC, Marks JH, Mandell JB; New York Breast Cancer Study Group. Breast and ovarian cancer risks due to inherited

mutations in BRCA1 and BRCA2. Science 2003:302(5645):643-46

Koli K, Keski-Oja J. 1alpha,25-dihydroxyvitamin D3 and its analogues down-regulate cell invasion-associated proteases in cultured malignant cells. Cell Growth Differ 2000:11(4):221-9

Koralek DO, Bertone-Johnson ER, Leitzmann MF, Sturgeon SR, Lacey JV Jr, Schairer C, Schatzkin A. Relationship between calcium, lactose, vitamin D, and dairy products and ovarian cancer. Nutr Cancer 2006:56(1):22-30.

Koskela P, Anttila T, Bjørge T, Brunsvig A, Dillner J, Hakama M, Hakulinen T, Jellum E, Lehtinen M, Lenner P, Luostarinen T, Pukkala E, Saikku P, Thoresen S, Youngman L, Paavonen J. Chlamydia trachomatis infection as a risk factor for invasive cervical cancer Int J Cancer 2000:85:35-9

Kotsopoulos J, Tworoger SS, Campos H, Chung FL, Clevenger CV, Franke AA, Mantzoros CS, Ricchiuti V, Willett WC, Hankinson SE, Eliassen AH. Reproducibility of plasma and urine biomarkers among premenopausal and postmenopausal women from the Nurses' Health Studies. Cancer Epidemiol Biomarkers Prev 2010:19(4):938-46.

Koushik A, Hunter DJ, Spiegelman D, Anderson KE, Arslan AA, Beeson WL, van den Brandt PA, Buring JE, Cerhan JR, Colditz GA, Fraser GE, Freudenheim JL, Genkinger JM, Goldbohm RA, Hankinson SE, Koenig KL, Larsson SC, Leitzmann M, McCullough ML, Miller AB, Patel A, Rohan TE, Schatzkin A, Smit E, Willett WC, Wolk A, Zhang SM, Smith-Warner SA. Fruits and vegetables and ovarian cancer risk in a pooled analysis of 12 cohort studies. Cancer Epidemiol Biomarkers Prev. 2005:14(9):2160-7.

Koushik A, Hunter DJ, Spiegelman D, Anderson KE, Buring JE, Freudenheim JL, Goldbohm RA, Hankinson SE, Larsson SC, Leitzmann M, Marshall JR, McCullough ML, Miller AB, Rodriguez C, Rohan TE, Ross JA, Schatzkin A, Schouten LJ, Willett WC, Wolk A, Zhang SM, Smith-Warner SA. Intake of the major carotenoids and the risk of epithelial ovarian cancer in a pooled analysis of 10 cohort studies. Int J Cancer 2006: 119;2148-54

Kurman RJ and Shih I-M. The origin and pathogenesis of epithelial ovarian cancer: a proposed unifying theory. Am J Surg Path 2010: 34;433-43

Kurman RJ, Shih I-M. Pathogenesis of ovarian cancer: lessons from morphology and molecular biology and their clinical implications. Int J Gynecol Pathol 2008:27(2):151-60.

Kushi LH, Mink PJ, Folsom AR, Anderson KE, Zheng W, Lazovich D, Sellers TA. Prospective study of diet and ovarian cancer. Am J Epidemiol 1999:149(1):21-31.

La Vecchia C. Oral contraceptives and ovarian cancer: an update, 1998-2004. Eur J Cancer Prev 2006:15(2):117-24

Lacey JV Jr, Brinton LA, Leitzmann MF, Mouw T, Hollenbeck A, Schatzkin A, Hartge P. Menopausal hormone therapy and ovarian cancer risk in the National Institutes of Health-AARP Diet and Health Study Cohort. J Natl Cancer Inst 2006:98(19):1397-405

Lacey JV Jr, Mink PJ, Lubin JH, Sherman ME, Troisi R, Hartge P, Schatzkin A, Schairer C. Menopausal hormone replacement therapy and risk of ovarian cancer. JAMA 2002:288(3):334-41

Langseth H, Hankinson SE, Siemiatycki J, Weiderpass E. Perineal use of talc and the risk of ovarian cancer. J Epidemiol Community Health 2008: 62; 358-60

Lappe JM, Travers-Gustafson D, Davies KM, Recker RR, Heaney RP. Vitamin D and calcium supplementation reduces risk: results of a randomized trial. Am J Clin Nutr 2007:85:1586-91

Larsson SC, Giovannucci E, Wolk A. Dietary folate intake and incidence of ovarian cancer: the Swedish Mammography Cohort. J Natl Cancer Inst. 2004:96(5):396-402.

Lefkowitz ES, Garland CF. Sunlight, vitamin D, and ovarian cancer mortality rates in US women. Int J Epidemiol 1994:23(6):1133-6.

Lehmann B, Meurer M. Vitamin D metabolism. Dermatol Ther 2010 ;23(1):2-12

Li H, Stampfer MJ, Hollis JB, Mucci LA, Gaziano JM, Hunter D, Giovannucci EL, Ma J. A prospective study of plasma

vitamin D metabolites, vitamin D receptor polymorphisms, and prostate cancer. PLoS Med. 2007:4(3):e103.
Lips P. Vitamin D physiology. Prog Biophys Mol Biol 2006:92(1):4-8
Liu SM, Koszewski N, Lupez M, Malluche HH, Olivera A, Russell J. Characterization of a response element in the 5_-flanking region of the avian (chicken) PTH gene that mediates negative regulation of gene transcription by 1,25-dihydroxyvitamin D3 and binds the vitamin D3 receptor. Mol Endocrinol 1996:10:206–15
Lomri A, Baron R.1,25-dihydroxyvitamin D3 regulates the transcription of carbonic anhydrase IImRNAin avian myelomonocytes. Proc Natl Acad Sci 1992: 89:4688–92
Love-Schimenti CD, Gibson DF, Ratnam AV, Bikle DD. Antiestrogen potentiation of antiproliferative effects of vitamin D3 analogues in breast cancer cells. Cancer Res 1996: 56(12):2789-94.
Lu Z, Chen TC, Holick MF. Influence of season and time of day on the synthesis of vitamin D3: In: Holick MF, Kligman A, eds. Proceedings of the Biologic Effect of Sunlight Symposium. Berlin: Walter De Gruyter and Co, 1992:48-52.
Lukanova A, Kaaks R. Endogenous hormones and ovarian cancer. Epidemiology and current hypothesis. Cancer Epidemiol Biomark Prev 2005:14(1):98-107.
Lundin E, Dossus L, Clendenen T, Krogh V, Grankvist K, Wulff M, Sieri S, Arslan AA, Lenner P, Berrino F, Hallmans G, Zeleniuch-Jacquotte A, Toniolo P, Lukanova A. C-reactive protein and ovarian cancer: a prospective study nested in three cohorts (Sweden, USA, Italy). Cancer Causes Control 2009: 20(7):1151-9.
Lurie G, Wilkens LR, Thompson PJ, McDuffie KE, Carney ME, Terada KY, Goodman MT. Vitamin D receptor gene polymorphisms and epithelial ovarian cancer risk. Cancer Epidemiol Biomarkers Prev 2007:16(12):2566-71.
Lynch HT, Casey MJ, Snyder CL, Bewtra C, Lynch JF, Butts M, Godwin AK. Hereditary ovarian carcinoma: heterogeneity, molecular genetics, pathology, and management. Mol Oncol 2009:3(2):97-13.
MacLaughlin JA, Anderson RR, Holick MF. Spectral character of sunlight modulat es photosynthesis of previtamin D3 an d its photoisomers in human skin. Scie nce 1982:216(4549): 1001-03
Malabanan A, Veronikis IE, Holick MF. Redefining vitamin D insufficiency. Lancet 1998;:351(9105):805-6
Mantell DJ, Owens PE, Bundred NJ, Mawer EB, Canfield AE. 1 alpha,25-dihydroxyvitamin D(3) inhibits angiogenesis in vitro and in vivo. Circ Res. 2000:87(3):214-20.
Mathiasen IS, Sergeev IN, Bastholm L, Elling F, Norman AW, Jäättelä M. Calcium and calpain as key mediators of apoptosis-like death induced by vitamin D compounds in breast cancer cells. J Biol Chem 2002: 277(34):30738-45.
Matsuoka, L. Y., Wortsman, J., Haddad, J. G. & Hollis, B. W. In vivo threshold for cutaneous synthesis of vitamin D3. J Lab Clin Med 1989:114:301–5
McCarty MF. Parathyroid hormone may be a cancer promoter- an explanation for the decrease in cancer risk associated with ultraviolet light, calcium and vitamin D. Med Hypotheses 2000:54(3):475-82
McCluggage WG. My approach to and thoughts on the typing of ovarian carcinomas. J Clin Pathol 2008: 61(2):152-63.
McConkey DJ and Orrenius S. The role of calcium in the regulation of apoptosis. Biochem Biophys Res Commun 1997:239:357-66
McSorley MA, Alberg AJ, Allen DS, Allen NE, Brinton LA, Dorgan JF, Pollak M, Tao Y, Helzlsouer KJ. C-reactive protein concentrations and subsequent ovarian cancer risk. Obstet Gynecol 2007: 109(4):933-41.
Mehta RG, Mehta RR. Vitamin D and cancer. J Nutr Biochem 2002:13(5):252-64.
Mellamby E, An experimental investigation on rickets, Lancet 1919: 4985; 407-12
Miettinen S, Ahonen MH, Lou YR, Manninen T, Tuohimaa P, Syvälä H, Ylikomi T. Role of 24-hydroxylase in vitamin D3 growth response of OVCAR-3 ovarian cancer cells. Int J Cancer 2004:108(3):367-73.
Miracle-McMahill HL, Calle EE, Kosinski AS, Rodriguez C, Wingo PA, Thun MJ, Heath CW Jr. Tubal ligation and fatal ovarian

cancer in a large prospective cohort study. Am J Epidemiol 1997:145(4):349-57.

Mitchell KO, El-Deiry WS. Overexpression of c-Myc inhibits p21WAF1/CIP1 expression and induces S-phase entry in 12-O-tetradecanoylphorbol-13-acetate (TPA)-sensitive human cancer cells. Cell Growth Differ 1999:10(4):223-30.

Mittal KR, Zeleniuch-Jacquotte A, Cooper JL, Demopoulos RI. Contralateral ovary in unilateral ovarian carcinoma, a search for preneoplastic lesions. Int. J. Gynecol Pathol 1993: 12: 59-63

Mizoue T. Ecological study of solar radiation and cancer mortality in Japan. Health Phys 2004:87(5):532-8.

Mørch LS, Løkkegaard E, Andreasen AH, Krüger-Kjaer S, Lidegaard O. Hormone therapy and ovarian cancer. JAMA 2009:302(3):298-305.

Morrison NA, Qi JC, Tokita A, Kelly PJ, Crofts L, Nguyen TV, Sambrook PN, Eisman JA. Prediction of bone density from vitamin D receptor alleles. Nature 1994: 367(6460):284-7

Muehlemann M, Miller KD, Dauphinee M, Mizejewski GJ. Review of Growth Inhibitory Peptide as a biotherapeutic agent for tumor growth, adhesion, and metastasis. Cancer Metastasis Rev 2005:24;441-67

Mullin GE, Dobs A. Vitamin d and its role in cancer and immunity: a prescription for sunlight. Nutr Clin Pract 2007:22(3):305-22

Nagpal S, Na S, Rathnachalam R. Noncalcemic actions of vitamin D receptor ligands. Endocr Rev 2005: 26(5):662-87.

Naora H. Developmental patterning in the wrong context: the paradox of epithelial ovarian cancers. Cell cycle 2005:8;1033-35

Napoli JL, Fivizzani MA, Schnoes HK, DeLuca HF. Synthesis of vitamin D5: its biological activity relative to vitamins D3 and D2. Arch Biochem Biophys 1979:197(1):119-25.

Navarro Silvera SA, Jain M, Howe GR, Miller AB, Rohan TE. Carotenoid, vitamin A, vitamin C, vitamin E intake and risk of ovarian cancer: a prospective study. Cancer Epidemiol Biomarkers Prev 2006:15(2);395-7.

Navarro Silvera SA, Jain M, Howe GR, Miller AB, Rohan TE. Dietary folate consumption and risk of ovarian cancer: a prospective cohort study. Eur J Cancer Prev. 2006:15(6):511-5.

Ness RB and Cottreau C. Possible role of ovarian epithelial inflammation in ovarian cancer. J Natl Cancer Inst 1999: 91: 1459-67.

Ness RB, Cramer DW, Goodman MT, Kjaer SK, Mallin K, Mosgaard BJ, Purdie DM, Risch HA, Vergona R, Wu AH. Infertility, fertility drugs, and ovarian cancer: a pooled analysis of case-control studies. Am J Epidemiol. 2002:155(3):217-24.

Nicosia SV, Saunders BO, Acevedo-Duncan ME, Setrakian S, Degregorio R. Biopathology of ovarian mesothelium. In:Familiari G, Makabe S, Motta PM (eds) Ultrastructure of the Ovary.Kluwer Academic Publishers, Boston, MA, 1991, pp 287–310.

Norman AW. From vitamin D to hormone D: fundamentals of the vitamin D endocrine system essential for good health. Am J Clin Nutr 2008:88(2):491S-9S.

Ocke MC, Shrijver J, Obermann-De Boer GL, Bloemberg B.P, Haenen G.R et al: Stability of blood (pro)vitamins during 4 years of storage at -20°C: consequences for epidemiologic research. J Clin Epi 1995:48: 1077-85.

Ono Y, Suzuki A, Kotake M, Zhang X, Nishiwaki-Yasuda K, Ishiwata Y, Imamura S, Nagata M, Takamoto S, Itoh M. Seasonal changes of serum 25-hydroxyvitamin D and intact parathyroid hormone levels in a normal Japanese population. J Bone Miner Metab 2005:23(2):147-51.

Pálmer HG, González-Sancho JM, Espada J, Berciano MT, Puig I, Baulida J, Quintanilla M, Cano A, de Herreros AG, Lafarga M, Muñoz A. Vitamin D(3) promotes the differentiation of colon carcinoma cells by the induction of E-cadherin and the inhibition of beta-catenin signaling. J Cell Biol 2001:23;154(2):369-87.

Parikh SJ, Edelman M, Uwaifo GI, Freedman RJ, Semega-Janneh M, Reynolds J, Yanovski JA. The relationship between obesity and serum 1,25-dihydroxyvitamin D concentrations in healthy adults. J Clin Endocrinol Metab 2004:89(3):1196-9

Park SY, Cooney RV, Wilkens LR, Murphy SP, Henderson BE, Kolonel LN. Plasma 25-hydroxyvitamin D and prostate cancer risk: the multiethnic cohort. Eur J Cancer. 2010:46(5):932-6.

Park Y, Leitzmann MF, Subar AF, Hollenbeck A, Schatzkin A. Dairy food, calcium, and risk of cancer in the NIH-AARP Diet and Health Study. Arch Intern Med 2009:169(4):391-401.

Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin 2005:55(2):74-108

Perez-Roger I, Kim SH, Griffiths B, Sewing A, Land H. Cyclins D1 and D2 mediate myc-induced proliferation via sequestration of p27(Kip1) and p21(Cip1). EMBO J 1999:18(19):5310-20.

Permuth-Wey J and Sellers TA. Epidemiology of ovarian cancer. In: M.Verma(ed.), Methods of Molecular Biology, Cancer Epidemiology, vol 472 2009

Pinette KV, Yee YK, Amegadzie BY, Nagpal S Vitamin D receptor as a drug discovery target. Mini Rev Med Chem 2003:3:193–204.

Porojnicu A, Robsahm TE, Berg JP, Moan J. Season of diagnosis is a predictor of cancer survival. Sun-induced vitamin D may be involved: a possible role of sun-induced Vitamin D. J Steroid Biochem Mol Biol 2007:103(3-5):675-8.

Prat J. Pathology of the ovary. Philadelphia: Saunders: 2004

Prentice A, Goldberg GR, Schoenmakers I. Vitamin D across the lifecycle: physiology and biomarkers. Am J Clin Nutr 2008:88(2):500S-6S.

Prospective study of diet and ovarian cancer. Am J Epidemiol 1999:149(1):21-31.

Prufer K, Racz A, Lin GC, Barsony J. Dimerization with retinoid X receptor promotes nuclear localization and subnuclear targeting of vitamin D receptor. J Biol Chem 2000:275:41114–23.

Pukkala E, Andersen A, Berglund G, Gislefoss R, Gudnason V, Hallmans G, Jellum E, Jousilahti P, Knekt P, Koskela P, Kyyrönen PP, Lenner P, Luostarinen T, Löve A, Ogmundsdóttir H, Stattin P, Tenkanen L, Tryggvadóttir L, Virtamo J, Wadell G, Widell A, Lehtinen M, Dillner J. Nordic biological specimen banks as basis for studies of cancer causes and control--more than 2 million sample donors, 25 million person years and 100,000 prospective cancers. Acta Oncol 2007:46(3):286-307.

Pukkala E. Finland – cancer registration. In: Pukkala E, et al. Cancer atlas of Northern Europe. Cancer Society of Finland Publication 62, Helsinki 2001, pp. 50-51

Purdie DM, Bain CJ, Siskind V, Russell P, Hacker NF, Ward BG, Quinn MA, Green AC. Hormone replacement therapy and risk of epithelial ovarian cancer. Br J Cancer 1999:81(3):559-63.

Quelo I, Machuca I, Jurdic P. Identification of a vitamin D response element in the proximal promoter of the chicken carbonic anhydrase II gene. J Biol Chem 1998:273:10638–46

Ramasamy I. Recent advances in physiological calcium homeostasis. Clin Chem Lab Med 2006:44(3):237-73.

Resta L, Russo S, Colucci GA, Prat J. Morphologic precursors of ovarian epithelial tumours. Obstet. Gynecol 1993: 82: 181-6.

Riman T, Dickman PW, Nilsson S, Correia N, Nordlinder H, Magnusson CM, Persson IR. Risk factors for invasive epithelial ovarian cancer: results from a Swedish case-control study. Am J Epidemiol 2002:156(4):363-73

Risch HA, Howe GR. Pelvic inflammatory disease and the risk of epithelial ovarian cancer. Cancer Epidemiol Biomarkers Prev 1995:4: 447-51

Rosenblatt KA, Thomas DB. Lactation and the risk of epithelial ovarian cancer. The WHO Collaborative Study of Neoplasia and Steroid Contraceptives. Int J Epidemiol 1993:22(2):192-7

Rossing MA, Daling JR, Weiss NS, Moore DE, Self SG. Ovarian tumors in a cohort of infertile women. N Engl J Med 1994:331(12):771-76

Rossing MA, Tang MT, Flagg EW, Weiss LK, Wicklund KG. A case-control study of ovarian cancer in relation to infertility and the use of ovulation-inducing drugs. Am J Epidemiol 2004:160(11):1070-8

Roth LM. Recent advances in the pathology and classification of ovarian sex cord-stromal tumours. Int J Gynecol Pathol 2006:25;199-215

Salazar-Martinez E, Lazcano-Ponce EC, Gonzalez Lira-Lira G, Escudero-De los Rios P, Hernandez-Avila M. Nutritional determinants of epithelial ovarian cancer risk: a case-control study in Mexico. Oncology 2002:63(2):151-7.

Sankaranarayanan R, Ferlay J. Worldwide burden of gynaecological cancer: the size of the problem. Best Pract Res Clin Obstet Gynaecol 2006: 20(2): 207-25

Schildkraut JM, Moorman PG, Halabi S, Calingaert B, Marks JR, Berchuk A. Analgesic use and the risk of ovarian cancer. Epidemiology 2006: 17:104-7

Schouten LJ, Rivera C, Hunter DJ, Spiegelman D, Adami HO, Arslan A, Beeson WL, van den Brandt PA, Buring JE, Folsom AR, Fraser GE, Freudenheim JL, Goldbohm RA, Hankinson SE, Lacey JV Jr, Leitzmann M, Lukanova A, Marshall JR, Miller AB, Patel AV, Rodriguez C, Rohan TE, Ross JA, Wolk A, Zhang SM, Smith-Warner SA. Height, body mass index, and ovarian cancer: a pooled analysis of 12 cohort studies. Cancer Epidemiol Biomarkers Prev. 2008:17(4):902-12

Scully RE and Sobin LH. Histological typing of ovarian tumours. 2nd ed. International Histological Classification of Tumours, Berlin: Springer, 1999.

Scully RE, Young RH, Clement PB. Tumours of the ovary, maldeveloped gonads, fallopian tubes, and broad ligament. Atlas of tumour pathology. 3rd series. Fascicle 23. Washington, DC: Armed Forces Institute of Pathology; 1998

Scully RE. Early de novo ovarian cancer and cancer developing in benign ovarian lesions. Int J Gynecol Obstet 1995: 49[Suppl]:S9–S15

Seidman JD, Horkayne-Szakaly I, Haiba M, Boice CR, Kurman RJ, Ronnett BM. The histological type and stage distribution of ovarian cancer of surface epithelial origin. Int J Gynecol Pathol 2004;23(1):41–4.

Sergeev I. N. Calcium as a mediator of 1, 25-dihydroxyvitamin D3-induced apoptosis. J Steroid Biochem 2004:89:419-25.

Sherr CJ, Roberts JM. Inhibitors of mammalian G1 cyclin-dependent kinases. Genes Dev 1995:9(10):1149-63.

Shih IM, Kurman RJ. Ovarian tumorigenesis. A proposed model based on morphological and molecular genetic analysis. Am J Pathol 2004:164:1511–8.

Sit AS, Modugno F, Weissfeld JL, Berga SL, Ness RB. Hormone replacement therapy formulations and risk of epithelial ovarian carcinoma. Gynecol Oncol 2002 :86(2):118-23.

Slovik DM, Adams JS, Neer RM, Holick MF, Potts JT, Jr. Deficient production of 1,25-dihydroxyvitamin D in elderly osteoporotic patients. N Eng J Med 1981:305(7):372-4.

Smith ML, Kontny HU, Zhan Q, Sreenath A, O'Connor PM, Fornace AJ Jr. Antisense GADD45 expression results in decreased DNA repair and sensitizes cells to u.v.-irradiation or cisplatin. Oncogene 1996:13(10):2255-63.

Soslow RA. Histologic subtypes of ovarian carcinoma: an overview. . Int J Gynecol Pathol 2008: 27:161-74.

St.-Arnaud R, Arabian A, and Glorieux FH. Abnormal bone development in mice deficient for the vitamin D-24-hydroxylase gene. J Bone Miner Res 1996:11:S126.

Stamp TC, Round JM. Seasonal changes in human plasma levels of 25-hydroxyvitamin D. Nature 1974:247(442):563-5.

Steingrimsdottir L, Gunnarsson O, Indridason OS, Franzson L, Sigurdsson G. Relationship between serum parathyroid hormone levels, vitamin D sufficiency, and calcium intake. JAMA 2005:294(18):2336-41

Suda T, Ueno Y, Fujii K, Shinki T. Vitamin D and bone. J Cell Biochem;2002:88:259–66.

Sueblinvong T and Carney ME. Current understanding of the risk factors for ovarian cancer. Curr Treat Options Oncol 2009:10(1-2); 67-81.

Sutton AL, MacDonald PN. Vitamin D: more than a “bonea-fide” hormone. Mol Endocrinol 2003:17:777–91

Talerman A. Germ cell tumours of the ovary. In: Kurman RJ, ed. Blaustein’s pathology of the female genital tract. 4th ed. New York: Springer-Verlag, 1994:849-914.

Tamez S, Norizoe C, Ochiai K, Takahashi D, Shimojima A, Tsutsumi Y, Yanaihara N, Tanaka T, Okamoto A, Urashima M. Vitamin D receptor polymorphisms and prognosis of patients with epithelial

ovarian cancer. Br J Cancer 2009:101(12):1957-60.

Teppo L, Pukkala E, Lehtonen M. Data quality and quality control of a population-based cancer registry. Experience in Finland. Acta Oncol 1994:33:365–9.

Thomas MK, Lloyd-Jones DM, Thadhani RI, Shaw AC, Deraska DJ, Kitch BT, Vamvakas EC, Dick IM, Prince RL, Finkelstein JS. Hypovitaminosis D in medical inpatients. N Engl J Med 1998:338(12):777-83.

Titus-Ernstoff L, Perez K, Cramer DW, Harlow BW, Baron JA, Greenberg ER. Menstrual and reproductive risk factors in relation to ovarian cancer risk. Br J Cancer 2001:84(5):714-721

Thomson CA, Neuhouser ML, Shikany JM, Caan BJ, Monk BJ, Mossavar-Rahmani Y, Sarto G, Parker LM, Modugno F, Anderson GL. The role of antioxidants and vitamin A in ovarian cancer: results from the Women's Health Initiative. Nutr Cancer 2008:60(6):710-9.

Tian XQ, Chen TC, Matsuoka LY, Wortsm an J, Holick MF. Kinetic and thermody namic studies of the conversion of pre vitamin D3 to vitamin D3 in human ski n. J Biol Chem 1993: 268(20):14888-92.

Tobler A, Gasson J, Reichel H, Norman AW, Koeffler HP 1987 Granulocyte-macrophage colony-stimulating factor. Sensitive and receptor-mediated regulation by 1,25-dihydroxyvitamin D3 in normal human peripheral blood lymphocytes. J Clin Invest 1987:79:1700–05.

Tong WM, Hofer H, Ellinger A, Peterlik M, Cross HS. Mechanism of antimitogenic action of vitamin D in human colon carcinoma cells: relevance for suppression of epidermal growth factor-stimulated cell growth.Oncol Res 1999:11(2):77-84.

Toriola AT, Grankvist K, Agborsangaya CB, Lukanova A, Lehtinen M, Surcel H-M. Changes in pre-diagnostic C-reactive protein concentrations and ovarian cancer risk. Ann Oncol 2010 (in press)

Travis RC, Crowe FL, Allen NE, Appleby PN, Roddam AW, Tjønneland A, Olsen A, Linseisen J, Kaaks R, Boeing H, Kröger J, Trichopoulou A, Dilis V, Trichopoulos D, Vineis P, Palli D, Tumino R, Sieri S, Bueno-de-Mesquita HB, van Duijnhoven FJ, Chirlaque MD, Barricarte A, Larrañaga N, González CA, Argüelles MV, Sánchez MJ, Stattin P, Hallmans G, Khaw KT, Bingham S, Rinaldi S, Slimani N, Jenab M, Riboli E, Key TJ. Serum vitamin D and risk of prostate cancer in a case-control analysis nested within the European Prospective Investigation into Cancer and Nutrition (EPIC). Am J Epidemiol. 2009:169(10):1223-32.

Tung KH, Wilkens LR, Wu AH, McDuffie K, Hankin JH, Nomura AM, Kolonel LN, Goodman MT. Association of dietary vitamin A, carotenoids, and other antioxidants with the risk of ovarian cancer. Cancer Epidemiol Biomarkers Prev 2005:14(3):669-76.

Tuohimaa P, Pukkala E, Scélo G, Olsen JH, Brewster DH, Hemminki K, Tracey E, Weiderpass E, Kliewer EV, Pompe-Kirn V, McBride ML, Martos C, Chia KS, Tonita JM, Jonasson JG, Boffetta P, Brennan P. Does solar exposure, as indicated by the non-melanoma skin cancers, protect from solid cancers: vitamin D as a possible explanation. Eur J Cancer 2007:43(11):1701-12.

Tworoger SS, Gates MA, Lee IM, Buring JE, Titus-Ernstoff L, Cramer D, Hankinson SE. Polymorphisms in the vitamin D receptor and risk of ovarian cancer in four studies. Cancer Res 2009:69(5):1885-91.

Tworoger SS, Lee I-M, Buring JE, Rosner B, Hollis BW, Hankinson SE. Plasma 25-hydroxyvitamin D and 1,25 dihydroxyvitamin D and the risk of incident ovarian cancer. Cancer Epidemiol Biomarkers Prev 2007:16:783-8.

Uitterlinden AG, Fang Y, Van Meurs JB, Pols HA, Van Leeuwen JP. Genetics and biology of vitamin D receptor polymorphisms. Gene 2004:338(2):143-56.

van Dam RM, Snijder MB, Dekker JM, Stehouwer CD, Bouter LM, Heine RJ, Lips P. Potentially modifiable determinants of vitamin D status in an older population in the Netherlands. Am J Clin Nutr 2007:85(3):755-61.

Venn A, Watson L, Lumley J, Giles G, King C, Healy D. Breast and ovarian cancer incidence after infertility and in vitro

fertilisation. Lancet 1995:346(8981): 995-1000.

Vieth R. Why the optimal requirement for Vitamin D3 is probably much higher than what is officially recommended for adults. J Steroid Biochem Mol Biol 2004:89-90(1-5):575-9.

Waltz P, Chodick G. Assessment of ecological regression in the study of colon, breast, ovary, non-Hodgkin's lymphoma, or prostate cancer and residential UV. Eur J Cancer Prev 2008:17(3):279-86.

Wang JH. Vitamin D, lipid metabolism and prostate cancer. Academic dissertation presented at the University of Tampere, 2009

Webb AR, DeCosta BR, Holick MF. Sunlig ht regulates the cutaneous production of vitamin D3 by causing its photodegr adation. J Clin Endocrinol Metab 1989: 68(5):882-7

Webb AR. Who, what, where and when-influences on cutaneous vitamin D synthesis. Prog Biophys Mol Biol 2006:92(1):17-25.

Weiss NS, Cook LS, Farrow DC, Rosenblatt KA. Ovarian cancer. In: Schottenfeld D, Fraumeni FJ, editors. Cancer epidemiology and prevention. New York: Oxford University Press; 1996. p1040–57.

Whittemore AS, Harris R, Itnyre J. Characteristics relating to ovarian cancer risk: collaborative analysis of 12 US case-control studies. II. Invasive epithelial ovarian cancers in white women. Collaborative Ovarian Cancer Group. Am J Epidemiol 1992:136:1184 – 1203

Williams SD, Gershenson DM, Horowitz CJ, Scully RE. Ovarian germ cell tumours. In: Hoskins WJ, Perez CA, Young RC, eds. Principles and practice of gynaecological oncology. 2nd ed. Pennsylvania: Lippincott-Raven, 1997;987-1002.

Wittenberg J, Cook LS, Rossing MA, Weiss NS. Reproductive risk factors for mucinous and non-mucinous epithelial ovarian cancer. Epidemiology 1999:10(6):761-3.

Wolpowitz D, Gilchrest BA. The vitamin D questions: how much do you need and how should you get it? J Am Acad Dermatol 2006:54(2):301-17.

World Health Organization, International Agency for Research on Cancer. Vitamin D and cancer. IARC working group reports, volume 5, 2008.

Wortsman J, Matsuoka LY, Chen TC, Lu Z, Holick MF. Decreased bioavailability of vitamin D in obesity. Am J Clin Nutr 2000:72(3);690-3.

Yamamoto H, Miyamoto K, Li B, Taketani Y, Kitano M, Inoue Y, Morita K, Pike JW, Takeda E. The caudal-related homeodomain protein Cdx-2 regulates vitamin D receptor gene expression in the small intestine. J Bone Miner Res 1999:14(2):240-7.

Yanagisawa J, Yanagi Y, Masuhiro Y, Suzawa M, Watanabe M, Kashiwagi K, Toriyabe T, Kawabata M, Miyazono K, Kato S. Convergence of transforming growth factor-beta and vitamin D signaling pathways on SMAD transcriptional coactivators. Science 1999: 26;283(5406):1317-21.

Ylikomi T, Laaksi I, Lou YR, Martikainen P, Miettinen S, Pennanen P, Purmonen S, Syvälä H, Vienonen A, Tuohimaa P. Antiproliferative action of vitamin D. Vitam Horm 2002:64:357-406.

Zerwekh JE. The measurement of vitamin D: analytical aspects. Ann Clin Biochem 2004:41:272-81.

Zhang X, Jiang F, Li P, Li C, Ma Q, Nicosia SV, Bai W. Growth suppression of ovarian cancer xenografts in nude mice by vitamin D analogue EB1089. Clin Cancer Res 2005;11(1):323-8.

Zhang X, Nicosia SV, Bai W. Vitamin D receptor is a novel drug target for ovarian cancer treatment. Curr Cancer Drug Targets 2006:6(3):229-44.

Zheng W, Danforth KN, Tworoger SS, Goodman MT, Arslan AA, Patel AV, McCullough ML, Weinstein SJ, Kolonel LN, Purdue MP, Shu XO, Snyder K, Steplowski E, Visvanathan K, Yu K, Zeleniuch-Jacquotte A, Gao YT, Hankinson SE, Harvey C, Hayes RB, Henderson BE, Horst RL, Helzlsouer KJ. Circulating 25-hydroxyvitamin D and risk of epithelial ovarian cancer: Cohort Consortium Vitamin D Pooling Project of Rarer Cancers. Am J Epidemiol 2010:172(1):70.

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The Effects of Storage Time and Sampling Season on the Stability of Serum 25-Hydroxy Vitamin D and Androstenedione

Calypse Agborsangaya [a]; Adetunji T. Toriola [b]; Kjell Grankvist [c]; Heljia-Marja Surcel [b]; Katsiaryna Holl [a]; Seppo Parkkila [a]; Pentti Tuohimaa [a]; Annekatrin Lukanova [d]; Matti Lehtinen [ab]
[a] University of Tampere, Tampere, Finland [b] National Institute for Health and Welfare, Oulu, Finland [c] Umeå University, Umeå, Sweden [d] German Cancer Research Centre, Heidelberg, Germany

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The Effects of Storage Time and Sampling Season on the Stability of Serum 25-Hydroxy Vitamin D and Androstenedione

Calypse Agborsangaya*
University of Tampere, Tampere, Finland

Adetunji T. Toriola*
National Institute for Health and Welfare, Oulu, Finland

Kjell Grankvist
Umeå University, Umeå, Sweden

Heljia-Marja Surcel
National Institute for Health and Welfare, Oulu, Finland

Katsiaryna Holl, Seppo Parkkila, and Pentti Tuohimaa
University of Tampere, Tampere, Finland

Annekatrin Lukanova
German Cancer Research Centre, Heidelberg, Germany

Matti Lehtinen
University of Tampere, Tampere, Finland and National Institute for Health and Welfare, Oulu, Finland

Knowledge of the stability of serum samples stored in large biobanks is pivotal for reliable assessment of hormone-dependent disease risks. We studied the effects of sample storage time and season of serum sampling on the stability of 25-hydroxy vitamin D (25-OHD) and androstenedione in a stratified random sample of 402 women, using paired sera from the Finnish Maternity Cohort. Serum samples selected were donated between 6 and 24 yr ago. The storage time did not affect serum 25-OHD and androstenedione levels. However, there was a significant mean difference in the 25-OHD levels of sera withdrawn during winter (first sample) vs. during summer (second sample; –18.4 nmol/l, $P \leq 0.001$). Also at the individual level, there were significant differences in average 25-OHD levels between individuals with the paired sera taken at winter–winter compared with other alternatives (summer–winter, winter–summer, and summer–summer). The androstenedione levels showed no such differences. Long-term storage does not affect serum 25-OHD and androstenedione levels, but sampling season is an important determinant of 25-OHD levels. Stored serum samples can be used to study disease associations with both hormones. However, sampling season needs to be taken into account for 25-OHD by considering matching and stratification and, if possible, serial sampling.

INTRODUCTION

Vitamin D is a "steroid-like" hormone, which is produced photochemically from 7-dehydrocholesterol on exposure of the skin to ultraviolet (UV) light. It can also be obtained from foods such as cod liver oil, salmon, eggs, liver, and so forth. Pre-vitamin D exists in 2 forms, ergocalciferol (vitamin D_2, from plant sources) and cholecalciferol (vitamin D_3, from animal sources). Once absorbed, vitamin D_3 undergoes hydroxylation in the liver to form 25-hydroxyvitamin (OH) D_3 (25-OHD_3, which is further hydroxylated to 1,25-dihydroxyvitamin D_3 (1,25 OH_2D_3) in the kidney, breast, colon, and prostate (1,2). The traditional function of 25-OHD is to maintain skeletal integrity by regulating calcium and phosphorus homeostasis (3,4). Epidemiological studies have associated low serum 25-OHD levels with a wide variety of diseases such as diabetes, some cancers, asthma, multiple sclerosis, rheumatoid arthritis, cardiovascular diseases, and schizophrenia (5).

*The first two authors contributed equally to this publication.

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Address correspondence to Calypse Agborsangaya, Tampere School of Public Health, Sorinkatu 1, 33100 Tampere, Finland. Phone: +358 3 3551 4179. Fax: +358 3 3551 8057. E-mail: calypse.agborsangaya@uta.fi

Because of the wide range of genomic and nongenomic effects of 25-OHD and its metabolites, a number of studies are being conducted exploring the 25-OHD disease associations. Most of these studies have exploited serum samples that have been stored in biobanks for several years, even decades. Stored serum 25-OHD is unaffected by multiple freeze-thaw cycles (6) and is stable when stored at –20°C for at least 3 yr (7). The impact of longer storage time has not been thoroughly considered. However, a recent study reported that maternal sera frozen for 40 yr could be used to detect seasonal and racial differences in 25-OHD concentrations (8). The serum 25-OHD concentrations in the 40-yr-old sera were, however, lower than those of 2-yr-old sera.

Androstenedione is a steroid hormone produced from the adrenals and ovaries. It is primarily synthesized from dehydroepiandrosterone (DHEA) and later reversibly converted to testosterone (9). Previous studies to determine seasonal changes in androgen levels have been equivocal (10–13).

The action of 25-OHD is androgen dependent in some organs such as the prostrate (14,15). An experimental study revealed that in intact animals, prostatic growth was significantly inhibited by 25-OHD_3, but in castrated animals who have low androgens, 25-OHD had no effect on prostatic growth (16). At physiologic concentrations, 25-OHD_3 exhibits strong antiproliferative activity only in the presence of dihydrotestosterone (14).

Using paired serum samples that have been stored for periods ranging from between 6 to 24 yr at the Finnish Maternity Cohort (FMC), we sought to determine the effects of storage time and sampling season on serum 25-OHD and androstenedione concentrations.

MATERIAL AND METHODS

FMC

The FMC contains 1.3 million first trimester serum samples from approximately 98% of all pregnant Finnish women. The samples have been collected since 1983. The samples are collected at municipal maternity care units following an informed consent during the first trimester of pregnancy for screening of congenital infections. After the screening tests have been done, the remaining sample (1–3 ml volume of serum) is stored at –25°C in polypropylene cryo vials at the National Institute for Health and Welfare (formerly National Public Health Institute) in Oulu, Finland (17). Detailed demographic and reproductive history data are collected for each participant.

Sample Identification

The samples used for this study were randomly selected following the sampling applied in a previous study within the FMC (18). In that study, 10 random first pregnancy samples were retrieved for every other year from 1984 to 2002 (altogether 100 samples). For this study, we selected 4 comparable first pregnancy samples taking into account also the sampling season: two samples for the same season (summer) and two for the opposite season (winter), age (+/– 4 yr), and postal code (municipality). We defined winter as the 15th of December to the 15th of March and summer as the 1st of May to the 31st of August. A total of 453 eligible individuals were identified from the FMC database. Of the corresponding 453 samples, eventually 402 samples were selected to make 201 summer samples and 201 winter samples appropriately matched for postal code and age. The lowest numbers of samples were from 1988 (37), and the highest numbers were from 1990, 1992, and 1996, with 42 samples each. From the final list of the 402 individuals, 100 individuals who had a second pregnancy within 5 yr of the first pregnancy were identified. We randomly selected 40 such individuals whose first and second pregnancies were from the same season (summer/summer and winter/winter) and 40 such individuals whose first and second pregnancy samples were from different seasons (summer/winter and winter/summer). Complete paired sample sets were available for 78 individuals.

Sample Handling

The available, previously unthawed samples were aliquoted into two 160 μl aliquots for 25-OHD and androstenedione analysis. Samples with indication of hemolysis or visual signs of freeze drying were not eligible. One aliquot per sample was sent to the Department of Anatomy, University of Tampere, Tampere, Finland, for 25-OHD analysis and another aliquot to the Clinical Chemistry Laboratory, Department of Medical Biosciences, Umeå University, Umeå, Sweden, for androstenedione analysis.

Laboratory Methods

Quantification of 25-OHD was done using an 25-OHD IDS-radioimmunoassay from IDS Ltd (Boldon, UK. The manufacturer stated a specificity (% cross-reactivity) of 100% for 25-OHD_3; 75% for 25-OHD_2; 100% for 24, 25-OH_2D_3; and less than 0.01% or 0.3% for cholecalciferol (D_3) and ergocalciferol (D_2), respectively. The mean interassay CV was 4.0% for the first pregnancy samples and 3.4% for the second pregnancy samples at 25-OHD mean of 26.5 nmol/l and 103 nmol/l, respectively.

Determination of serum levels of androstenedione were performed at the Department of Medical Biosciences, University of Umeå, Umeå, Sweden. The steroid levels were measured by competitive chemiluminescent enzyme immunoassay (DPC Immulite 2000 Androstenedione Chemiluminescent EIA, UK). With control sera, the interassay CV was 16.9% at 2.2 ng/ml and 6.2% at 5.4 ng/ml.

The study was approved by the ethical committee of the National Institute for Health and Welfare, Finland.

Statistical Methods

We used SPSS (SPSS, Inc., Chicago, IL) version 14 for all statistical analysis. Serum 25-OHD was not normally

TABLE 1
Baseline characteristics of a stratified, random sample of women stemming from the Finnish Maternity Cohort with single and paired pregnancy samples

	1st Pregnancy Samples		1st and 2nd Pregnancy Samples	
Variable	Winter	Summer	Winter	Summer
Number (N)	163	161	38	40
Mean age (yr)	28.6	28.7	30.8	31.2
Mean storage time (yr)	14.9	14.9	13.2	12.3

distributed, and we performed a logarithmic transformation. Serum androstenedione was normally distributed. Clustered box plots were drawn for both and the duration of sample storage, stratified by sampling season. Pearson's partial correlation coefficient (r) was used to test for correlation between duration of sample storage and both 25-OHD and androstenedione. Independent sample t-test was used to assess the effect of sampling season on single hormone measurement, whereas paired sample t-test was used to assess the effect of the sampling season on paired hormone measurements.

RESULTS

The baseline characteristics (mean age, sample storage time) of the women who donated one or two pregnancy serum samples in different seasons were similar. For the first pregnancy samples, the mean and median ages at sample withdrawal were 28.7 and 29 yr, respectively (range = 22–38 yr). Mean duration of storage was 14.9 yr, ranging from 6 yr to 24 yr. For the second pregnancy samples, the mean and median ages were 31.0 and 30.4 yr, respectively (range = 25–40 yr). Their mean duration of storage was 12.8 yr, ranging from 4 to 22 yr (Table 1).

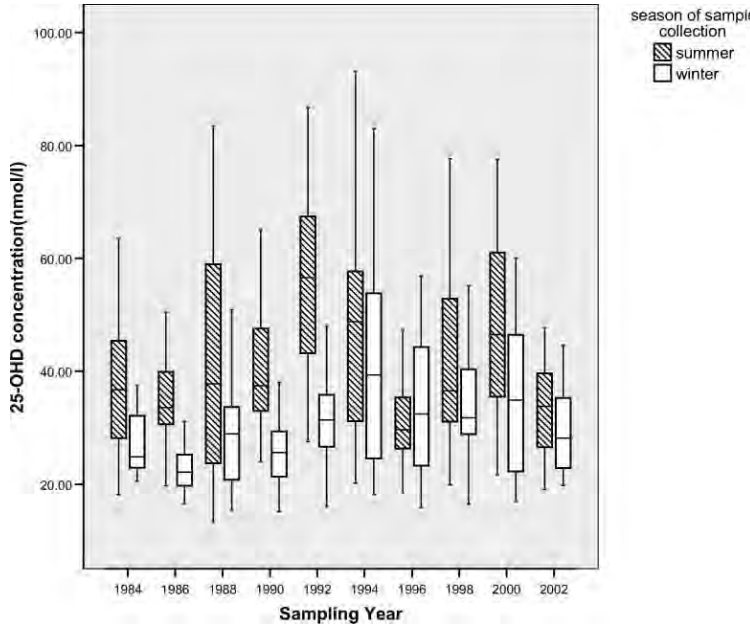

FIG. 1. Serum 25-hydroxy vitamin D (25-OHD; nmol/l) in a stratified, random sample of pregnant women (n = 402) stemming from the Finnish Maternity Cohort (1984–2002) by sampling season. The median serum 25-OHD levels are significantly higher in summer than in winter. The error bars represent the 25th (lower) and 75th (upper) percentiles.

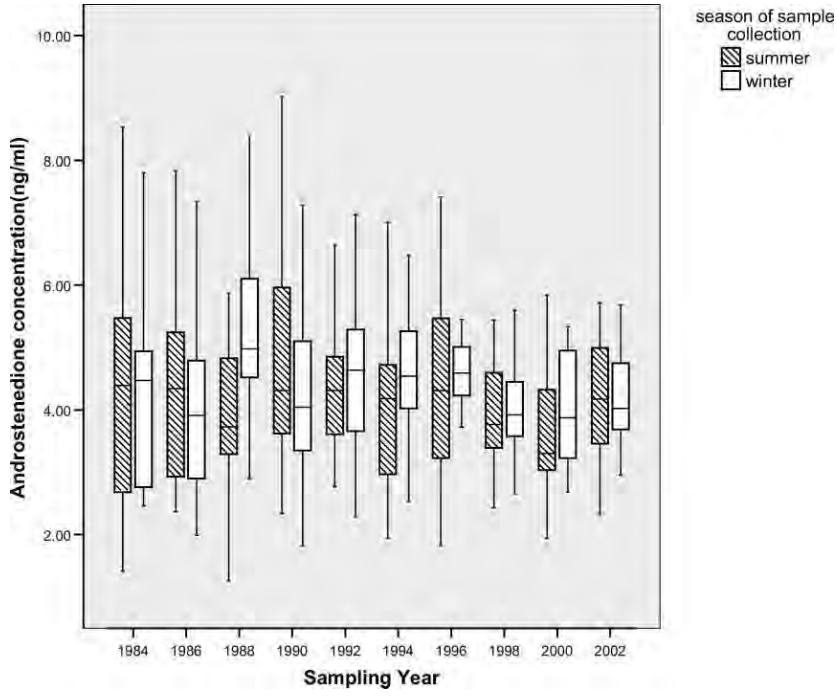

FIG. 2. Serum androstenedione (ng/ml) in a stratified, random sample of pregnant women stemming from the Finnish Maternity Cohort (1984–2002) by sampling season. The median serum androstenedione levels appear to be higher in winter than in summer. The error bars represent the 25th (lower) and 75th (upper) percentiles.

The highest average levels of serum 25-OHD were found in 1992 [44.8 nmol/l, 95% CI = 39.2–50.5) and 1994 (44.5 nmol/l, 95% CI = 38.8–50.2) and the lowest in 1996 (34.2 nmol/l, 95% CI = 30.4–38.0) and 2002 samples (34.7 nmol/l, 95% CI = 30.1–39.3). For the first pregnancy samples, mean serum 25-OHD levels were significantly lower in winter than in summer; 33.4 nmol/l (95% CI = 31.5–35.3) vs. 44.0 nmol/l (95% CI = 41.4–46.5; $P \leq 0.001$), respectively. This was true also for the second pregnancy samples; 39.8 nmol/l (95% CI = 46.7–56.5) vs. 51.6 nmol/l (95% CI = 34.5–45.0; $P \leq 0.001$), respectively. When stratified by the sampling season, the average annual serum 25-OHDlevels were lower in the winter than in the summer (Figs. 1 and 2). The mean androstenedione levels appeared to be higher in winter than in summer (mean difference $\mu_d = 0.3$ nmol/ml, 95% CI = 0.0–0.6; P value = 0.05).

Serum 25-OHD levels showed practically no correlation with storage time for the first pregnancy samples ($r_s = -0.08$, P value = 0.1) or for the second pregnancy samples ($r_s = -0.09$, P value = 0.5). Furthermore, there was no correlation between the storage time and serum 25-OHD levels after controlling for sampling age in either the first ($r_s = -0.02$, P value = 0.7) or in the second pregnancy samples ($r_s = -0.08$, P value = 0.51). Age adjusted androstenedione levels had no significant correlation with storage time ($r_s = 0.09$).

We found a highly significant mean difference in the 25-OHD levels of the paired sera withdrawn during winter (first sample) vs. during summer (second sample; mean difference $\mu_d = -18.4$ nmol/l; P value ≤ 0.001; Table 2). This was true (albeit to the opposite direction) also for individuals with the first sampling season at summer and the second sampling season at winter ($\mu_d = 9.4$ nmol/l; P value < 0.1; Table 2). Comparing the average serum 25-OHD levels for groups of individuals with different kinds of pairs of sampling seasons (identical or opposite seasons), we found significant differences when winter–winter samples were compared to all other types of sample pairs (Table 3). The highest differences were observed when comparing the average serum 25-OHD levels for winter–winter samples with summer–summer ($\mu_d = 16.6$ nmol/l, P value ≤ 0.001) and summer–winter samples ($\mu_d = 17.8$ nmol/l; P value ≤ 0.001). No significant differences were observed

TABLE 2
Mean differences in serum 25-OHD (nmol/l) in paired sera of pregnant Finnish women by season of sample withdrawal[a]

Seasons of Sample Withdrawal	Number (N)	Mean Differences Between 1st and 2nd Samples	95% Confidence Interval	P Value
Winter–Summer	19	−18.4	−25.05 to −11.68	≤ 0.001
Summer–Winter	15	9.4	−2.07 to 20.85	0.10
Summer–Summer	19	−1.9	−10.81 to 7.04	0.67
Winter–Winter	21	−5.1	−10.55 to 0.36	0.07

[a]Abbreviation is as follows: 25-OHD, 25-hydroxy vitamin D.

when similar comparisons were made for androstenedione levels (Table 3).

DISCUSSION

Long-term storage of serum samples in biobanks at –25°C had no effect on 25-OHD and androstenedione concentrations. Sampling season, however, had an effect. The average 25-OHD concentrations were significantly lower in single and paired samples taken during winter than in summer. If anything, androstenedione concentrations tended to show the opposite.

The results from our study confirm previous studies on vitamin D stability. Ocke et al. (7) analyzed serum vitamin D concentrations of the same individual at intervals during 4 yr and found approximately 10% difference (increase or decrease) in mean vitamin D concentrations at given time points. They concluded that the differences are most likely due to systematic differences in laboratory measurements. Bodnar et al. (8) compared 25-OHD concentrations in sera stored for 40 yr with those of sera stored for 2 yr in different individuals. Even though the mean 25-OHD concentrations in the 40-yr-old samples were lower than those of the 6-yr-old samples, the measurements were similar in both. Seasonal and racial differences in mean 25-OHD concentrations were found between both cohorts, implying that if there was any deterioration in 25-OHD detectability, it was similar across all the samples (8).

The first pregnancy samples of our cohort had been drawn in paired years between 1984 and 2002 (6–24 yr ago). We did not observe any correlation between serum 25-OHD concentrations and the sampling time. However, we observed a consistent statistically significant association between 25-OHD concentrations and the sampling season. Also in the paired samples of the same individuals, mean serum 25-OHD concentrations were always higher in summer than in winter, an observation that has been previously well documented in single sample analyses (8,19,20).

The highest mean serum 25-OHD concentrations were found in 1992, mainly due to very high summer levels of that year, an observation that also holds true for the other years with mean serum 25-OHD concentrations above 40 nmol/l. Likewise, the

TABLE 3
Differences in average serum androstenedione (ng/ml) and 25-OHD (nmol/l) of paired pregnancy samples of Finnish women with specific pregnancy history[a]

Sampling Season	Summer–Summer	Summer–Winter	Winter–Summer	Winter–Winter
	25-OHD, nmol/l (mean difference[b] with p-value)			
Summer–Summer		1.2(0.83)	−6.9(0.11)	16.6(0.001)[c]
Summer–Winter			−8.04(0.11)	17.8(0.001)[c]
Winter–Summer				9.7(0.02)[c]
Winter–Winter				
	Androstenedione ng/mL (mean difference[b] with p-value)			
Summer–Summer				
Summer–Winter	−0.3(0.4)			
Winter–Summer	−0.2(0.5)	0.1(0.8)		
Winter–Winter	−0.2(0.6)	−0.6(0.1)	−0.5(0.2)	

[a]Abbreviation is as follows: 25-OHD, 25-hydroxy vitamin D.
[b]Mean differences between averages of paired serum.
[c]Mean 25-OHD concentrations for Winter–Winter paired samples are significantly lower than other season pairs.

lowest total mean 25-OHD concentrations were found in 1996, the year with the lowest summer 25-OHD concentrations. The year 1996 was the only year with average summer 25-OHD concentrations less than 35 nmol/l. The low summer values in 1996 are probably due to the cold weather in 1996 (below the average for the century) and the fact that July 1996 was the wettest July in 100 yr in parts of Finland (21). Thus, the possibility of obtaining 25-OHD from sunlight was lower compared to other years.

The diet of Finnish pregnant women has been fortified with 25-OHD since February 2003 (22). Our study samples were predominantly taken before this date. Differences in serum 25-OHD levels can therefore be attributed to seasonal variation. This implies that summer 25-OHD status, which is a reflection of exposure to UV light from the sun is the most important determinant of 25-OHD status in our Nordic cohort. This is because at latitudes above 35°C (such as Finland), winter sunlight cannot stimulate cutaneous production of pre-25-OHD since most, if not all, of the UVB photons below 315 nm are absorbed by the ozone layer (1). Our ability to detect the low summer serum 25-OHD values in the 1996 samples also reinforces the fact that 25-OHD is stable if stored properly at –25°C for many years, and possible deterioration is random.

For sample pairs with identical sampling seasons, average 25-OHD levels for the second pregnancy samples were higher than the first pregnancy levels for both winter–winter and summer–summer pairs. These results are consistent with previous findings that pregnancy increases nutrition awareness. Szwajcer et al. (23) carried out interviews of 1-h duration with 60 women categorized in 5 groups: nulliparous, nonpregnant women; groups of first, second, and third trimester pregnant nulliparous women; and second pregnancy women in the first trimester. They found that pregnancy leads to increased nutritional awareness, which becomes a habit during the second pregnancy. The increased health conscience among pregnant women may lead to frequent sunlight exposure or increased dietary intake of foods rich in vitamin D, which could translate into higher levels of average 25-OHD for women with second pregnancies compared to the women with first pregnancies.

The androstenedione levels were somewhat higher in winter than in summer. We found this also in the maternity cohort samples used in Holl et al. study (24) (data not shown). These results are consistent with experimental studies (25,26). Sing and Krishna (25) determined seasonal changes in testosterone and androstenedione levels in adult rats and observed the highest androstenedione levels in winter (November–January). In an earlier study, Smith et al. (26) studied the effect of seasonal variability on the levels of androstenedione and testosterone in male blue foxes. They observed a steady increase in the levels of both hormones during winter months (November–March). Contrary to our findings, Bjørnerem et al (11) and Brambilla et al. (27) did not observe any seasonal variation in the serum androgens (DHEA and testosterone) in blood samples of adult males and females. DHEA is a precursor in synthesis of androstenedione, which in turn is reversibly converted to testosterone (8). Thus, seasonal stability in serum levels of androstenedione should correlate with the levels of DHEA and testosterone.

In prospective epidemiologic studies using biomarkers as quantitative estimates of past exposures and disease risk, serial samples may provide a more reliable estimate compared to static, single sample, estimates (28). Although there is compelling evidence on the difference between summer and winter levels of 25-OHD, to the best of our knowledge, no study has compared serial (paired) samples taken both in similar and different seasons. Our study shows that significant differences in 25-OHD levels due to sampling season exist also for paired samples, especially if the samples are drawn in winter compared to other seasons. We suggest that epidemiologic studies assessing 25-OHD levels and disease risk should appropriately stratify and match subparts by sampling season and use serial samples wherever possible to get better insight into the vitamin D status of cases and controls.

REFERENCES

1. Holick MF: Vitamin D: importance in the prevention of cancer, Type 1 diabetes, heart disease, and osteoporosis. *Am J Clin Nutr* **79,** 362–371, 2004.
2. Webb AR, Kline L, and Holick MF: Influence of season and latitude on the cutaneous synthesis of vitamin D3: exposure to winter sunlight in Boston and Edmonton will not promote vitamin D3 synthesis in human skin. *J Clin Endocrinol Met* **67,** 373–378, 1988.
3. Lips P: Vitamin D physiology. *Prog Biophys Mol Biol* **92,** 4–8, 2006.
4. Wolpowitz D and Gilchrest BA: The vitamin D questions: how much do you need and how should you get it. *J Am Acad Dermatol* **54,** 301–317, 2006.
5. Holick MF: Vitamin D deficiency. *N Engl J Med* **357,** 266–281, 2007.
6. Antonuicci DM, Black DM, and Sellmeyer DE: Serum 25-Hydroxyvitamin is unaffected by multiple freeze–thaw cycles. *Clin Chem* **51,** 258–261, 2005.
7. Ocke MC, Shrijver J, Obermann-De Boer GL, Bloemberg BP, Haenen GR, et al: Stability of blood (pro)vitamins during 4 years of storage at –20°C: consequences for epidemiologic research. *J Clin Epidemiol* **48,** 1077–1085, 1995.
8. Bodnar LM, Catov JM, Wisner KL, and Klebanoff MA: Racial and seasonal differences in 25-hydroxyvitamin D detected in maternal sera frozen for over 40 years. *Br J Nutr* **23,** 1–7, 2008.
9. Ming-qiu HU and Yan-qun NA: Metabolism of adrenal androgen and its impacts on prostate cancer after castration. *Chin Med J* **121,** 369–374, 2008.
10. Svartberg J, Jorde R, Sundsfjord J, Bønaa KH, and Barrett-Connor E: Seasonal variation of testosterone and waist to hip ratio in men: the Tromsø Study. *J Clin Endocrinol Metab* **88,** 3099–3104, 2003.
11. Bjørnerem Å, Straume B, Øian P, and Berntsen GK: Seasonal variation of estradiol, follicle stimulating hormone, and dehydroepiandrosterone sulfate in women and men. *J Clin Endocrinol Metab* **91,** 3789–3802, 2006.
12. Andersson AM, Carlsen E, Petersen JH, and Skakkebaek NE: Variation in levels of serum inhibin B, testosterone, estradiol, luteinizing hormone, follicle-stimulating hormone, and sex hormone-binding globulin in monthly samples from healthy men during a 17-month period: possible effects of seasons. *J Clin Endocrinol Metab* **88,** 932–937, 2003.
13. Maes M, Mommen K, Hendrickx D, Peeters D, D'Hondt P, et al.: Components of biological variation, including seasonality, in blood concentrations of TSH, TT3, FT4, PRL, cortisol and testosterone in healthy volunteers. *Clin Endocrinol* **46,** 587–598, 1997.

14. Lou Y-R and Tuohimaa P: Androgen enhances the anti-proliferative activity of 25-OHD$_3$by suppressing 24-hydoxylase expression in LNCaP cells. *J Steroid Biochem Mol Biol* **92,** 307–315, 2004.
15. Zhao X-Y, Peehl, DM, Navone NM, and Feldman D: 1 alpha, 25-dihydroxy vitamin D3 inhibits prostate cancer cell growth by androgen dependent and androgen independent mechanisms. *Endocrinology* **141,** 2548–2556, 2000.
16. Leman ES, Arlotti JA, Dhir R, and Getzenberg RH: 25-OHD and androgen regulation of prostatic growth. *J Cell Biochem* **90,** 138–147, 2003.
17. Koskela P, Anttila T, Bjørge T, Brunsvig A, Dillner J, et al.: Chlamydia trachomatis infection as a risk factor for invasive cervical cancer. *Int J Cancer* **85,** 35–39, 2000.
18. Holl K, Lundin E, Kaasila M, Grankvist K, Afanasyeva Y, et al.: Effect of long-term storage on hormone measurements in samples from pregnant women: the experience of the Finnish Maternity Cohort. *Acta Oncol* **47,** 406–412, 2008.
19. Nakamura K, Nashimoto M, and Yamamoto M: Summer/winter differences in the serum 25-hydroxyvitamin D_3 and parathyroid hormone levels of Japanese women. *Int J Biometeorol* **44,** 186–189, 2000.
20. Harris SS and Dawson-Hughes B: Seasonal changes in plasma 25-hydroxyvitamin D concentrations of young American black and white women. *Am J Clin Nutr* **67,** 1232–1236, 1998.
21. Kløve B and Bengtsson L: Runoff generation in a plough-drained cutover fen in central Finland. *J Hydrology* **218,** 157–168, 1999.
22. Laaksi IT, Ruohola JP, Ylikomi TJ, Auvinen A, Haataja RI, et al.: 25-OHD fortification as public health policy: significant improvement in 25-OHD status in young Finnish men. *Eur J Clin Nutr* **60,** 1035–1038, 2006.
23. Szwajcer EM, Hiddink GJ, Maas L, Koelen MA, and van Woerkum CMJ: Nutrition-related information-seeking behaviors of women trying to conceive and pregnant women: evidence for the life course perspective. *Fam Pract* **25,** 99–104, 2008.
24. Holl K, Lundin E, Surcel HM, Grankvist K, Koskela P, et al.: Endogenous steroid hormone levels in early pregnancy and risk of testicular cancer in the offspring: a nested case-referent study. *Int J Cancer* **124**, 2923–2928, 2009.
25. Singh UP and Krishna A: Seasonal changes in circulating testosterone and androstenedione concentration and their correlation with the anomalous reproductive pattern in the male Indian sheath-tailed bat, *Taphozous longimanus*. *J Exp Zool* **287,** 54–61, 2000.
26. Smith AJ, Mondain-Monval M, Møller OM, Scholler R, and Hansson V: Seasonal variations of LH, prolactin, androstenedione, testosterone and testicular FSH binding in the male blue fox (Alopex lagopus). *J Reprod Fertil* **74,** 449–458, 1995.
27. Brambilla DJ, O'Donnell AB, Matsumoto AM, and McKinlay JB: Lack of seasonal variation in serum sex hormone levels in middle-aged to older men in the Boston area. *J Clin Endocrinol Metab* **92,** 4224–4429, 2007.
28. Woltering EA, Hilton RS, Zolfoghary CM, Thomson J, Zietz S, et al.: Validation of serum versus plasma measurements of chromogranin a levels in patients with carcinoid tumors: lack of correlation between absolute chromogranin a levels and symptom frequency. *Pancreas* **33,** 250–254, 2006.

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Serum 25-hydroxyvitamin D and the risk of ovarian cancer ☆

Adetunji T. Toriola [a,b,], Helja-Marja Surcel [a], Calypse Agborsangaya [b], Kjell Grankvist [c], Pentti Tuohimaa [d], Paolo Toniolo [e], Annekatrin Lukanova [f], Eero Pukkala [b,g], Matti Lehtinen [a,b]*

[a] *National Institute for Health and Welfare, Finland*
[b] *Tampere School of Public Health, University of Tampere, Finland*
[c] *Department of Medical Biosciences, Umeå University, Umeå, Sweden*
[d] *Department of Anatomy, University of Tampere, Finland*
[e] *Department of Obstetrics and Gynecology, New York University School of Medicine, New York, USA*
[f] *Department of Cancer Epidemiology, German Cancer Research Centre, Heidelberg, Germany*
[g] *Finnish Cancer Registry, Institute for Statistical and Epidemiological Cancer Research, Helsinki, Finland*

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ABSTRACT

Introduction: Ecological and experimental studies suggest that vitamin D may be associated with a reduced risk of ovarian cancer. In this study, we sought to determine the risk of developing ovarian cancer according to serum 25-hydroxyvitamin D (25-OHD) concentrations assessed on average 5 years before the diagnosis.

Methods: We conducted a population-based longitudinal case–control study nested within the Finnish Maternity Cohort (FMC) which contains serum samples of virtually all pregnant women in Finland since 1983. Among them, 201 ovarian cancers diagnosed within 10 years of serum sampling were randomly selected as cases for this study. For each case, we selected two controls matched for age, parity and sampling season (±4 weeks) and one control matched for age and parity but for the opposite sampling season (6 months ± 4 weeks).

Results: The relative risks (estimated as odds ratio, OR) for ovarian cancer comparing the lowest quintile to the highest quintile of serum 25-OHD concentration were 1.8 (95% CI 0.9–3.5) among controls matched for the same season, and 1.1 (95% CI 0.6–2.2) among controls matched for the opposite season. The OR among women with insufficient (<75 nmol/L) serum 25-OHD concentration was 2.7 (95% CI 1.0–7.9, lower limit, 0.95) compared to that among those with sufficient (⩾75 nmol/L) serum 25-OHD concentration. No differences in the point estimates were observed between serous or mucinous histological subtypes of ovarian cancer.

Conclusion: Overall, we did not observe a significant association between serum 25-OHD concentrations and the risk of ovarian cancer. However, we found evidence suggestive of an increased risk among women with low to insufficient serum 25-OHD concentrations.

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* *Corresponding author:* Address: National Institute for Health and Welfare, PL 310, FIN-90101, Finland. Tel.: +358 20610 6210; fax: +358 206106251.
E-mail addresses: Adetunji.toriola@uta.fi, Adetunji.toriola@thl.fi (A.T. Toriola).

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1. Introduction

Ovarian cancer is the most lethal gynaecological malignancy and the fourth leading cause of cancer deaths among women worldwide.[1] About 191,000 new cases are diagnosed annually,[2] with the highest incidence rates found in Caucasians in northern and western Europe and in North America.[2] Despite its considerable burden to cancer mortality in women, very little is known about its aetiology.[1]

Ecological studies have suggested that there may be an association between solar ultraviolet B radiation and risk of ovarian cancer.[3–6] It has been postulated that the protective effects of sunlight on ovarian cancer are mediated via vitamin D, which is a steroid-like hormone mainly produced through the action of sunlight on the skin. Of the six ecological studies published regarding ovarian cancer mortality rates and solar radiation, four[3–6] reported higher mortality rates with lower regional sunlight whereas two observed no relationship.[7,8] Solar ultraviolet B (UVB) and high solar irradiance were also shown to be inversely associated with incidence rates of ovarian cancer in a worldwide study[9] while there was no association in another multinational study.[10] Likewise, epidemiological studies examining the association of dietary vitamin D and ovarian cancer have been equivocal.[11–14] However, one recent study that evaluated the association of plasma vitamin D and ovarian cancer risk reported a beneficial effect among certain subgroups.[15]

Vitamin D, by inducing cellular differentiation and apoptosis, inhibiting cellular proliferation, invasiveness and angiogenesis possesses anti-carcinogenic properties.[16] Vitamin D exerts its action through the vitamin D receptor (VDR) and VDRs are present in both normal ovarian tissues and ovarian cancer cells.[17–19]

In this nested case–control study, we examined the relationship between serum vitamin D concentration and risk of ovarian cancer exploiting the resources of the nationwide Finnish Maternity Cohort (FMC), established in 1983.[20]

2. Materials and methods

2.1. *Finnish Maternity Cohort*

We carried out a case–control study nested within the Finnish Maternity Cohort (FMC). The FMC was established by the National Institute for Health and Welfare, Finland in 1983.[20] Following an informed consent, first trimester blood samples are withdrawn from pregnant women at the municipal maternity care units to screen for intrauterine infections. After the screening has been done, the remaining sample (1–3 mL of serum) is stored at −25 °C in polypropylene cryo vials in a well-protected biorepository at the National Institute for Health and Welfare in Oulu. More than 98% of pregnant women in Finland have donated blood samples to the cohort since 1983 and currently over 1.3 million samples are kept in storage. Each year about 60,000 new serum samples are added to the repository.

2.2. *Identification of cases and controls*

Incident ovarian cancer cases were identified by the population-based Finnish Cancer Registry (FCR). All cancer cases diagnosed in Finland since 1953 are reported to the FCR (reporting mandatory since 1961). The coverage of the FCR is virtually complete with no losses to follow-up.[21] Every resident of Finland has a unique personal identity code that is also used in official health registries such as the FMC and FCR. Our study cohort was record linked with the cancer registry data by using the personal identity code.

Two-hundred and one random ovarian cancer cases diagnosed within 10 years of serum sampling were selected for this study. If a case had donated more than one sample within this 10-year period, the sample donated closest to cancer diagnosis was selected. Time between serum donation and cancer diagnosis ranged between 2 years and 10 years with a median of 5 years. Among the 201 cancer cases, 195 had histological confirmation. Of the 195 with histological confirmation, 76 (39%) were serous, 70 (35.9%) mucinous, 15 (7.7%) sex cord stromal tumours, 11 (5.6%) endometrioid, 9 (4.6%) germ cell tumours, 5 (2.6%) clear cell tumours and 9 (4.6%) others. The tumours were left-sided in 74 (45.4%) cases, right-sided in 66 (40.5%) cases and bilateral in 23 (14.1%) cases.

The cases were matched with two sets of controls. The first set of controls consisted of women from the FMC who were alive and free of cancer at the time of diagnosis of the case and were matched for (i) age at sample withdrawal ±1 year, (ii) parity and (iii) date of index blood sampling ±4 weeks (same season as case). For each case, 2 controls were selected and 400 serum samples were available for the laboratory analysis. The second set of controls consisted of women from the cohort, who were alive and free of cancer at the time of diagnosis of the cases and whose serum samples were taken at the opposite seasons to the cases (approximately six months ± 4 weeks apart). These controls were matched for (i) age at sample withdrawal ±1 year and (ii) parity. One control per case was selected. In all, 201 cases, 398 same season controls and 199 opposite season controls were available.

All study samples were previously unthawed. The study was approved by the ethical committee of the National Institute for Health and Welfare, Finland.

2.3. *Laboratory analysis*

Quantification of 25-hydroxyvitamin D (25-OHD) was done at the Department of Medical Biosciences, University of Umeå, Umeå, Sweden using a 25-OHD radioimmunoassay (RIA) from IDS Ltd., Boldon, UK. The manufacturer stated a specificity (% cross-reactivity) of 100% for 25-OHD3, 75% for 25-OHD2, 100% for 24, 25-OH2D3, and less than 0.01% and 0.3% for cholecalciferol (D3) and ergocalciferol (D2), respectively. The within, between and total coefficients of variations (CVs) of the assay were 7.8%, 9.6% and 12.4% at level 28.4 nmol/L 25-OHD, and 4.1%, 7.4% and 8.5% at 107 nmol/L 25-OHD, respectively. Case and control samples belonging to each other study set were assayed together, ordered randomly and labelled to mask case–control status.

2.4. *Statistical analysis*

Two of the samples had 25-OHD concentrations of less than 2 nmol/L, which were considered to be outliers and set to missing. Descriptive data of cases and controls with regard

to age at sample withdrawal and cancer diagnosis, age at last full term pregnancy, number of pregnancies, gestational days and bench lag-time were recorded.

Quintile cut-off points were determined using 25-OHD concentrations of the controls. Quintiles of 25-OHD concentrations were used to estimate the relative risk of ovarian cancer and their 95% confidence intervals (CIs) by conditional logistic regression and are expressed as odds ratio (OR). The multivariate model was adjusted for age at last full term pregnancy and bench lag-time (days between sample withdrawal and freezing it for storage). Separate analyses were conducted for the main different histological subgroups of ovarian cancer available vis-a-vis, serous and mucinous tumours using cases and controls that were matched for the same season. Risk of ovarian cancer was also compared among women with serum 25-OHD levels above 75 nmol/L and those with serum 25-OHD levels below 75 nmol/L.[22,23] All statistical analyses were performed using SPSS 15 for windows (SPSS Inc., Chicago, IL). Two-sided $p < 0.05$ was considered statistically significant.

3. Results

The age range for cases and controls was from 17.5 to 44 years. The median ages at the time of serum sampling were 31.5 and 31.4 years for cases and controls, respectively. The median duration between serum sampling and cancer diagnosis was 5 years. The median number of pregnancies was 2 with a range of 1–8 for cases and 1–10 for controls. Median serum 25-OHD concentrations were 35.0, 35.8 and 34.3 nmol/L among cases, same season controls and opposite season controls, respectively (Table 1).

We first compared the relative risk of ovarian cancer by quintile groups of serum 25-OHD among cases and controls whose serum samples were taken within the same season and had been matched for age and parity. Low levels of 25-OHD appeared to be associated with increased risk of ovarian cancer. The strongest association did, however, not reach statistical significance (1st quintile versus 5th quintile: OR, 1.8, CI 0.9–3.6; $p_{trend} = 0.26$) age at last full term pregnancy and bench lag-time adjusted (Table 2). Women with insufficient serum vitamin D concentrations had a threefold increased risk of ovarian cancer compared to women with sufficient serum concentrations (OR 2.8, 95% CI 1.0–7.9, lower limit 0.95, p-value = 0.06) (Table 3). Different point estimates were obtained when control samples from opposite seasons were used. The point estimates were closer to unit risk (1st quintile versus 5th quintile: OR, 1.1, CI 0.6–2.2; $p_{trend} = 0.49$) (Table 2).

No significant associations were observed between quintiles of 25-OHD concentrations and ovarian cancer risk when the analyses were carried out for the two main histological groups. Comparing the 1st to the 5th quintiles, the ORs were 1.3 (95% CI 0.7–3.2) for serous tumours, and 1.2 (95% CI 0.4–3.1) for mucinous tumours. Stratification for seasonality did not show any further differences (data not shown).

4. Discussion

In this longitudinal population-based nested case–control study, we observed no significant relationship between serum 25-hydroxyvitamin D concentrations and the risk of ovarian cancer. However, there appeared to be a tendency to increased risk among those who had low or insufficient serum 25-hydroxyvitamin D concentrations compared to those who had sufficient concentrations.

Perhaps, one of the reasons why an association between 25-OHD and ovarian cancer was not apparent using the quintile distribution of 25-OHD was the generally relatively low serum 25-OHD concentrations in the cohort. Recently, due to a greater understanding of the interplay between vitamin D and its regulatory hormones, there have been discussions on what constitutes vitamin D deficiency.[22–24] A 25-OHD concentration of less than 50 nmol/L is considered to represent vitamin D deficiency, and that between 50 and 72 nmol/L is considered to represent relative insufficiency while concentrations above 75 nmol/L are thought to indicate vitamin D sufficiency.[22] Using these cut-off values, we found evidence suggestive of an increased risk of ovarian cancer among women with insufficient serum vitamin D concentrations compared with women with sufficient vitamin D concentrations. This supports results from the experimental studies which suggest that high vitamin D concentrations may be needed to prevent against ovarian cancer.[25,26]

The results from our study are similar to those of another recent prospective study by Tworoger and colleagues.[15] The authors did not observe any association between plasma 25-

Table 1 – Baseline characteristics of the study population who donated serum samples to the Finnish Maternity Cohort between 1983 and 2006.

	Cases (n = 201) Median (range)	Control[a] (n = 398) Median (range)	Control[b] (n = 199) Median (range)
Age at serum sampling	31.5 (17.5–43.2)	31.5 (17.8–43.9)	31.4 (18.9–43.6)
Age at last full term pregnancy	32.7 (17.5–50.1)	33.6 (17.8–50.9)	33.2 (18.9–51.2)
Number of pregnancies	2 (1–8)	2 (1–10)	2 (1–10)
Lag-time to cancer diagnosis, years	5 (0.5–9.8)		
Duration of gestation, days	74.0 (40–89)	76.5 (33–91)	81.0 (35–87)
Bench lag-time, days	2.0 (0–12)	3.0 (0–9)	3.0 (0–9)
25-OHD concentration (10th and 90th percentiles), nmol/L	35.0 (20.3, 56.4)	35.8 (22.9, 61.3)	34.3 (21.5, 65.4)

a Same season controls. Serum samples were taken within 1 month to those of cases.

b Opposite season controls. Serum samples were taken during the opposite season to those of cases, i.e. approximately 6 months ± 4 weeks apart.

Table 2 – Relative risks (odds ratio, OR, with 95% confidence interval, CI) of ovarian cancer by quintile of serum vitamin D concentration in fertile aged Finnish women followed up for up to 10 years after serum withdrawal.

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	P_{trend}
Among cases and controls who donated serum in same season (±4 weeks)[a]						
Quintile values, nmol/L	<26.4	26.4–32.9	32.9–39.9	39.9–53.1	⩾53.1	0.26
n, cases, control	46/79	41/81	41/81	44/78	28/79	
OR, crude	1.6 (0.9–2.9)	1.4 (0.8–2.6)	1.4 (0.8–2.5)	1.6 (0.9–2.7)	1.0 (reference)	
OR, adjusted[b]	1.8 (0.9–3.5)	1.6 (0.8–3.1)	1.5 (0.8–2.8)	1.6 (0.9–2.9)	1.0 (reference)	
Among cases and controls who donated serum in opposite seasons (6 months ± 4 weeks)[a]						
Quintile values, nmol/L	<25.3	25.3–32.2	32.2–38.8	38.8–51.9	⩾51.9	0.49
n, cases, control	42/39	36/41	41/39	41/39	41/40	
OR, crude	1.1 (0.6–2.0)	1.3 (0.7–2.3)	1.1 (0.6–2.0)	1.1 (0.6–2.0)	1.0 (reference)	
OR, adjusted[b]	1.1 (0.6–2.2)	1.3 (0.6–2.5)	1.2 (0.6–2.3)	1.2 (0.6–2.3)	1.0 (reference)	

a Cases and controls were also matched for parity and age.
b Adjusted for age at last full term pregnancy and bench lag-time.

Table 3 – Relative risks (odds ratio, OR, with 95% confidence interval, CI) of ovarian cancer in women with sufficient serum vitamin D concentrations compared with those of fertile aged Finnish women with insufficient serum vitamin D concentrations followed up for up to 10 after serum withdrawal.

	Cases	Controls	OR	OR, adjusted (95% CI)	*P*-value
Sufficient ⩾ 75 nmol/L (reference)[b]	4	21	1.0		0.06
Insufficient (<75 nmol/L)	196	377	2.8	2.7 (1.0[c]–7.9)	

a Analysis carried out among cases and controls matched for age, parity and season of serum donation (±4 weeks).
b Refs. 22,23.
c Lower limit, 0.95.

hydroxyvitamin D and ovarian cancer risks in their overall analysis but women with adequate versus inadequate 25-hydroxyvitamin D levels had a decreased risk of borderline significance (RR, 0.7; 95% CI, 0.4–1.1). In addition, Tworoger and colleagues reported a stronger effect among overweight women. Unfortunately, data on body weight were not available for the women in our sub-sample of the FMC to explore this hypothesis.

The ovary together with other tissues such as the kidney, breast, colon and prostate express the enzyme, 1 alpha-hydroxylase, (1aOHase).[25] This enzyme converts 25-OHD to 1, 25-$(OH)_2$D, thus, the biologically active form of vitamin D is available locally in the ovaries and other tissues that express the enzyme. Since the ovaries also express VDR[18,19] and the effects of vitamin D are exerted through the VDR, it is biologically plausible that vitamin D may have effects on the ovaries.

Ovarian cancer is a heterogeneous disease. The histological subtypes have different risk factors (both genetic and environmental) and dissimilar epidemiological and clinical characteristics.[27] In our study, we did not observe any differences in risk related to vitamin D concentration by histology. Apart from serous and mucinous cancers, the other histological subgroups, however, had very few cases.

The following limitations of our study need to be taken into account: (i) oral contraceptive use increases serum 25-OHD concentrations[28,29]; and use of oral contraceptives also protects against ovarian cancer.[30] Hence, use of oral contraceptives is a confounder in the association between 25-OHD and ovarian cancer. While this was controlled for by sample withdrawal during 12th to 14th week of pregnancy (when use of oral contraceptive must have been stopped for sometime), we did not have information on the use of oral contraceptives among our study subjects. (ii) Our study subjects were limited to fertile women in their reproductive ages who were matched for parity. Therefore we do not know if similar results will be obtained in infertile and/or post-menopausal women since they were not represented in our study.

Serum 25-OHD concentration is a reflection of endogenous synthesis taking place in the skin under the influence of UVB irradiation and exogenous production from dietary intake.[22,31] Vitamin D first undergoes hydroxylation in the liver to 25-hydroxyvitamin D (25-OHD) before undergoing further hydroxylation to 1, 25-dihydroxyvitamin D (1, 25-$(OH)_2$D) in the kidneys.[22] While 1, 25-$(OH)_2$D is the most biologically active form of vitamin D, 25-OHD is considered as the best gauge of individual vitamin D status,[22,31,32] making measurement of serum/plasma 25-OHD concentrations the ideal way to determine vitamin D status.

Strengths of our study include (i) its population-based nature; the FMC covers 98% of pregnant Finnish women.[20] (ii) Its prospective nature; the blood samples were collected before cancer diagnosis. Furthermore, the study samples were also collected before the start of vitamin D supplementation among pregnant women in Finland. In addition, we were able to explore the possible effects of matching for season of blood collection on the results. The reason why we selected the opposite season control group was to determine how much variation in risk estimates can occur when such controls are used as compared to when same season controls are used.

We believe that this may have important repercussions on studies examining vitamin D/disease associations especially in serial sample studies. In this study, deliberate opposite season controls showed lower, close to unit risk estimates when compared to season-matched controls. This could mean that if an effect exists, not taking into account the input of seasonality probably confounds the estimation. In epidemiological studies ascertaining vitamin D disease associations, adequate matching of cases and controls for time of sample collection and/or statistical control is essential.

In conclusion, although we did not observe statistically significant association between serum 25-OHD concentrations and risk of ovarian cancer, low serum 25-OHD concentrations may be associated with an increased risk of developing the disease. As few modifiable factors are known to reduce ovarian cancer risk, further prospective data on vitamin D and ovarian cancer are of interest.

Conflict of interest statement

None declared.

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REFERENCES

1. Barnes MN, Grizzle WE, Clinton JG, Patridge EE. Paradigms for primary prevention of ovarian carcinoma. *CA Cancer J Clin* 2002;**52**:216–25.
2. Runnebaum IG, Stickeler E. Epidemiological and molecular aspects of ovarian cancer risk. *J Cancer Res Clin Oncol* 2001;**127**:73–9.
3. Freedman DM, Dosemeci M, McGlynn K. Sunlight and mortality from breast, ovarian, colon, prostate, and non-melanoma skin cancer: a composite death certificate based case–control study. *Occup Environ Med* 2002;**59**:257–62.
4. Grant WB. An estimate of premature cancer mortality in the US due to inadequate doses of solar ultraviolet-B radiation. *Cancer* 2002;**59**:257–62.
5. Lefkowitz ES, Garland CF. Sunlight, Vitamin D and ovarian cancer mortality rates in US women. *Int J Epidemiol* 1994;**23**:1133–6.
6. Grant WB. Ecological studies of solar UV-B radiation and cancer mortality rates. *Recent Results Cancer Res* 2003;**164**:371–7.
7. Mizoue T. Ecological studies of solar radiation and cancer mortality in Japan. *Health Phys* 2004;**87**:532–8.
8. Robsahm TE, Trelti S, Dahlback A, Moan J. Vitamin D3 from sunlight may improve the prognosis of breast, colon, and prostate cancer (Norway). *Cancer Causes Control* 2004;**15**:149–58.
9. Garland CF, Morh SB, Gorham ED, Grant WB, Garland FC. Role of ultraviolet B irradiance and vitamin D in prevention of ovarian cancer. *Am J Prev Med* 2006;**31**(6):512–4.
10. Tuohimaa P, Pukkala E, Scelo G, et al. Dose solar exposure, as indicated by the non-melanoma skin cancers, protect from solid cancers: vitamin D as a possible explanation. *Eur J Cancer* 2007;**43**:1701–12.
11. Bidoli E, La Vecchia C, Talamani R, et al. Micronutrients and ovarian cancer: a case–control study in Italy. *Ann Oncol* 2001;**12**:1589–93.
12. Salazar-Martinez E, Lacano-Ponce EC, Gonzalez Lira-Lira G, Escudero-De los Rios P, Hernandez-Avila M. Nutritional determinants of epithelial ovarian cancer risk: a case–control study in Mexico. *Oncology* 2002;**63**:151–7.
13. Gerkinger JM, Hunter DJ, Spiegelman D, et al. Diary products and ovarian cancer: a pooled analysis of 12 cohort studies. *Cancer Epidemiol Biomarkers Prev* 2006;**15**:364–72.
14. Goodman MT, Wu AH, Tung KH, et al. Association of diary products, lactose, and calcium with the risk of ovarian cancer. *Am J Epidemiol* 2002;**156**:148–57.
15. Tworoger SS, Lee I-M, Buring JE, Rosner B, Hollis BW, Hankinson SE. Plasma 25-hydroxyvitamin D and 1, 25 dihydroxyvitamin D and the risk of incident ovarian cancer. *Cancer Epidemiol Biomarkers Prev* 2007;**16**:783–8.
16. Giovanucci E. The epidemiology of vitamin D and cancer incidence and mortality: a review (United States). *Cancer Causes Control* 2005;**16**:83–95.
17. Ahonen MH, Zhuang YH, Aine R, Ylikomi T, Tuohimaa P. Androgen receptor and vitamin D receptor in human ovarian cancer: growth stimulation and inhibition by ligands. *Int J Cancer* 2000;**86**:40–6.
18. Agic A, Xu H, Altgassen C, et al. Relative expression of 1, 25-dihydroxyvitamin D3 receptor, vitamin D1 alpha-hydroxylase, vitamin D 24-hydroxylase, and vitamin 25-hydroxylase in endometriosis and gynaecological cancers. *Reprod Sci* 2007;**14**:486–97.
19. Norman AW. From vitamin D to hormone D: fundamentals of the vitamin D endocrine system essential for good health. *Am J Clin Nutr* 2008;**88**(Suppl.):491s–9s.
20. Koskela P, Antilla T, Bjørge T, et al. Chlamydia trachomatis infection as a risk factor for invasive cervical cancer. *Int J Cancer* 2000;**85**:35–9.
21. Teppo L, Pukkala E, Lehtonen M. Data quality and quality control of a population-based cancer registry. Experience in Finland. *Acta Oncol* 1994;**33**:365–9.
22. Hollick MF. Vitamin D deficiency. *N Engl J Med* 2007;**357**: 266–81.
23. Vieth R, Bischoff-Ferrari H, Boucher BJ, et al. The urgent need to recommend an intake of vitamin D that is effective. *Am J Clin Nutr* 2007;**85**:649–50.
24. Dawson-Hughes B, Heaney RP, Holick MF, Lips P, Meunier PJ, Vieth R. Estimates of optimal vitamin D status. *Osteoporos Int* 2005;**16**:713–6.
25. Miettinen S, Ahonen MH, Lou Y-L, et al. Role of 24-hydroxylase in vitamin D3 growth response of OVCAR-3 ovarian cancer cells. *Int J Cancer* 2004;**108**:367–73.
26. Rashid SF, Mountford JC, Gombart AF, Campbell MJ. 1 alpha, 25-dihydroxyvitamin D(3) displays divergent growth effects in both normal and malignant cells. *Steroids* 2001;**66**:433–40.
27. Chen VW, Ruiz B, Killeen JL, et al. Pathology and classification of ovarian tumours. *Cancer* 2003;**97**:2631–42.
28. Harris SS, Dawson-Hughes B. The association of oral contraceptive use with plasma 25-hydroxyvitamin D levels. *J Am Coll Nutr* 1998;**17**(3):282–4.
29. Thane CW, Bates CJ, Prentice A. Oral contraceptives and nutritional status in adolescent British girls. *Nutr Res* 2002;**22**:449–62.

30. Collaborative Group on Epidemiological Studies of Ovarian Cancer, Beral V, Doll R, Hermon C, Peto R, Reeves G. Ovarian cancer and oral contraceptives: collaborative reanalysis of data from 45 epidemiological studies including 23 257 women with ovarian cancer and 87 303 controls. *Lancet* 2008;**371**(9609):303–14.
31. Mullin GE, Dobs A. Vitamin D and its role in cancer and immunity: a prescription for sunlight. *Nutr Clin Pract* 2007;**22**:305–22.
32. International Agency for Research on Cancer, World Health Organization. *Vitamin D and cancer*, vol. 5. IARC working group report, 2008.

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Independent and joint effects of serum 25-hydroxyvitamin D and calcium on ovarian cancer risk: A prospective nested case–control study

Adetunji T. Toriola [a,b,], Helja-Marja Surcel [a], Agborsangaya Calypse [a,b], Kjell Grankvist [c], Tapio Luostarinen [d], Annekatrin Lukanova [e], Eero Pukkala [b,d], Matti Lehtinen [a,b]*

[a] *National Institute for Health and Welfare, Finland*
[b] *Tampere School of Public Health, University of Tampere, Finland*
[c] *Department of Medical Biosciences, Umeå University, Umeå, Sweden*
[d] *Finnish Cancer Registry, Institute for Statistical and Epidemiological Cancer Research, Helsinki, Finland*
[e] *Department of Cancer Epidemiology, German Cancer Research Centre, Heidelberg, Germany*

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ABSTRACT

Introduction: Ovarian cancer has very few known modifiable risk factors but dietary studies suggest a role for vitamin D and calcium in the prevention of ovarian cancer. Thus, we investigated the association between pre-diagnostic serum calcium and 25-hydroxyvitamin D (25-OHD) on the risk of later development of ovarian cancer.

Methods: We conducted a population-based nested case–control study within the Finnish Maternity Cohort (FMC). The cohort subset comprised 172 ovarian cancer cases with 172 matched controls (age ± 1 year, parity and season of blood donation ± 2 weeks).

Results: We observed a significant inverse association between calcium and ovarian cancer risk. Relative risk (estimated as odds ratio, OR) comparing the highest quartile to the lowest quartile was significantly decreased; 0.41 [95% confidence interval (CI) 0.19–0.85, P-trend 0.004]. Even though a comparable association between 25-OHD and ovarian cancer did not reach statistical significance (OR 0.57, 95% CI 0.26–1.24, P-trend 0.07), having sufficient (>75 nmol/L) serum 25-OHD levels compared to insufficient serum 25-OHD was associated with a significantly decreased risk of ovarian cancer (OR 0.32; 95% CI 0.12–0.91, *p*-value 0.03). No synergistic protective interaction between high levels of calcium and 25-OHD against ovarian cancer was observed, the joint effect being just multiplicative.

Conclusion: Calcium and vitamin D act independently to reduce the risk of ovarian cancer. The decreased risk of ovarian cancer associated with pre-diagnostic serum calcium and vitamin D needs to be evaluated further for possible new insights into ovarian cancer prevention.

* *Corresponding author:* Address: National Institute for Health and Welfare, PL 310, FIN-90101, Finland. Tel.: +358 20610 6210; fax: +358 20610 6251.
E-mail addresses: Adetunji.toriola@uta.fi, Adetunji.toriola@thl.fi (A.T. Toriola).
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1. Introduction

Ecological, dietary and experimental studies suggest that vitamin D may be associated with reduced risk of ovarian cancer.[1–4] So far, only three epidemiological studies have directly explored this association by relating serum 25-hydroxyvitamin D (25-OHD) to risk, but the results were not conclusive.[5–7] Nevertheless, in two of the three studies, women with sufficient vitamin D concentrations tended to have lower risk of ovarian cancer overall.[5,6] Thus, more studies to characterise a possible association of ovarian cancer with vitamin D and explore the effect of additional factors that may modify risk are of interest.

The classical function of vitamin D is to facilitate the intestinal absorption of calcium but evidence is mounting to suggest that the actions of vitamin D and calcium on physiologic processes may be synergistic.[8,9] High extracellular calcium can modulate vitamin D metabolism in favour of increased conversion to 1,25-dihydroxyvitamin D (the active form of vitamin D) which in turn may up-regulate the expression of the calcium receptor and increase intestinal calcium absorption.[9] In support, a randomised clinical trial recently demonstrated that while calcium supplementation reduced the risk of all-cause cancer among women, the protective effect was more pronounced among women with combined calcium and vitamin D supplementation.[10] Along the same lines, the protective effect of high dietary calcium intake on colorectal cancer incidence was strongest in subjects with highest vitamin D intake in a large pooled analysis of 10 cohort studies.[11]

The independent role of serum calcium in ovarian cancer has not been explored so far. While some studies have observed an inverse association of ovarian cancer with dietary intake of calcium, others have observed positive or no associations.[12–17] Nevertheless, the relationship between serum calcium concentrations and dietary calcium intake is complex. Serum calcium concentrations are tightly regulated and there is very little correlation between it and dietary calcium intake.[18] To the best of our knowledge, no previous study has investigated the effect of serum calcium and the joint effects of serum calcium and vitamin D levels on ovarian cancer risk. The aim of this study was to evaluate possible independent and joint effects of vitamin D and calcium on the risk of ovarian cancer.

2. Material and methods

2.1. Finnish Maternity Cohort

This study is a prospective, population based case–control study nested within the Finnish Maternity Cohort (FMC). The FMC was established by the National Institute for Health and Welfare (formerly National Public Health Institute), Finland, in 1983.[19] Following an informed consent, first trimester blood samples are withdrawn from pregnant women at the municipal maternity care units to screen for intrauterine infections. After the screening has been done, the remaining sample (1–3 mL of serum) is stored in polypropylene cryo vials at −25 °C in a well-protected biorepository at the National Institute for Health and Welfare, Oulu. More than 98% of pregnant women in Finland have donated blood samples to the cohort since 1983 and currently about 1.6 million samples are kept in storage. Each year about 60,000 new serum samples are added to the repository.

2.2. Identification of cases and controls

Incident ovarian cancer cases were identified by the population-based Finnish Cancer Registry (FCR). All cancer cases diagnosed in Finland since 1953 are reported to the FCR (reporting mandatory since 1961). The coverage of the FCR is virtually complete with no losses to follow-up.[20] Every resident of Finland has a unique personal identity code that is also used in official health registries like the FMC and the FCR. These codes were used as the linkage key. Ovarian cancer cases who had donated serum samples to the FMC more than once, at least 1 year apart, before cancer diagnosis were selected for this study. Of the 215 cases that fulfilled these criteria, those who donated their last sample within 1 year before the cancer diagnosis and those who had been selected for a previous study of ovarian cancer and vitamin D within this cohort[5] were excluded, leaving 172 cases. There were 166 cases with histological confirmation of which 68 (41%), 60 (36%) and 15 (9%) were serous, mucinous and endometroid cancers, respectively.

Eligible controls were women from the FMC who were alive and free of cancer at the time of diagnosis of the index case and who also had donated at least two serum samples during different pregnancies to the FMC. The controls were matched for (i) age at sample withdrawal ± 1 year, (ii) parity and (iii) date of index blood sampling ±2 weeks for both sets of samples. One control with paired samples was selected for every case. For this study, the first set of serum samples for cases and controls was used.

The study was approved by the ethical committee of the National Institute for Health and Welfare, Finland.

2.3. Laboratory analysis

Quantification of 25-OHD was performed at the Department of Medical Biosciences, University of Umeå, Umeå, Sweden, using a 25-OHD radioimmunoassay (RIA) from IDS Ltd., Boldon, United Kingdom. The manufacturer stated a specificity (% cross-reactivity) of 100% for 25-OHD3, 75% for 25-OHD2, 100% for 24, 25-OH2D3 and less than 0.01% or 0.3% for cholecalciferol (D3) and ergocalciferol (D2), respectively. The within, between and total coefficients of variation (CV) of the assay were 4.3%, 17.1% and 17.7% at level 32.9 nmol/L, respectively. Serum 25-OHD concentrations were defined as follows: (i) sufficient > 75 nmol/L, (ii) relative insufficient 50–75 nmol/L and (iii) insufficient or deficient <50 nmol/L.[21] Outliers were defined as concentrations exceeding three times the interquartile range and were set to missing (n = 1 for 25-OHD).

Quantification of serum calcium was performed at the Clinical Chemistry Laboratory, Östersunds Hospital, Östersund, Sweden, using the Roche/Hitachi cobas c system analyzer (Roche Diagnostics GmbH, D68298 Mannheim, Germany). The total coefficient of variation (CV) of the assay was 2.4% at level 2.0 mmol/L. Case and control samples belonging to the same study set were assayed together, but

ordered randomly and labelled to mask case–control status. Three samples had insufficient volume to perform the laboratory assays.

2.4. Statistical analysis

Descriptive statistics are presented as mean (standard deviation) for all other data and median and percentiles for calcium and 25-OHD.

Quartile cut-off points for both 25-OHD and calcium were determined using the distribution among the controls. Conditional logistic regression was used to calculate odds ratio with 95% confidence interval (OR with 95% CI) for ovarian cancer in the different quartiles of 25-OHD and calcium using the lowest quartiles as the reference category. Tests for trend were calculated using continuous scale of the variables, log-transformed for 25-OHD because the overall distribution was slightly skewed even though the season-specific distributions were normal. The effect of adjustment for age at first full-term pregnancy, age at last full-term pregnancy, gestational day at blood donation and region of residence (North, South, West, East and Central Finland) were investigated in multivariate models, and variables that altered risk estimates by more than 5% were retained in the final models (age at first full-term pregnancy and region of residence). Mutual adjustments of vitamin D models for calcium concentrations and vice versa were also performed but the results are very identical to those obtained without the mutual adjustments and are thus not presented. Secondary analyses were carried out excluding cases (and their matched controls) whose cancers were diagnosed within 1–3 years of serum sampling (analysis was also done excluding cases diagnosed within 1–2 years of sampling but the results were identical to those obtained excluding cases diagnosed within 1–3 years and thus not presented) and for women with sufficient/insufficient serum vitamin D (>75 nmol/L).[21]

To determine the possible synergistic effects of 25-OHD and calcium, we categorised the subjects into four groups based on each individual's combined 25-OHD and calcium status. (i) Low 25-OHD /low calcium – women with both 25-OHD and calcium categories within the 1st 3 quartiles, i.e. (25-OHD Q1, Q2, Q3 + calcium Q1, Q2, Q3). This represented the reference category, (ii) high 25-OHD/low calcium – women whose 25-OHD were within the 4th quartile but whose calcium were within the 1st 3 quartiles (25-OHD Q4 + calcium Q1, Q2, Q3), (iii) low 25-OHD/high calcium – women with 25-OHD within the 1st 3 quartiles but calcium within the 4th quartile (25-OHD Q1, Q2, Q3 + calcium Q4) and (iv) high 25-OHD/high calcium – both 25-OHD and calcium were within the 4th quartiles (25-OHD Q4 + calcium Q4). Testing for effect modification was done with a likelihood ratio test to compare two nested models, by considering the difference between the model-specific scaled deviances. All statistical analyses were performed using SPSS 18 for windows (SPSS, Inc., Chicago IL). Two sided $p < 0.05$ was considered statistically significant.

3. Results

Mean age at serum sampling for both cases and controls was the same (29.9 years). Likewise ages at first full-term and last full-term pregnancies and number of pregnancies were almost identical for both cases and controls. The mean lag time between serum sampling and cancer diagnosis was 6.4 years (range 1–13.5 years). Median 25-OHD concentrations were 39.2 nmol/L (90th percentile 65.4) and 40.0 nmol/L (90th percentile 73.4); while median calcium concentrations were 2.3 mmol/L and 2.4 mmol/L for cases and controls, respectively (Table 1). There was no correlation between serum 25-OHD and calcium concentrations among cases (rs = −0.02, *p*-value 0.82) and controls (rs = 0.07, *p*-value 0.39).

Mean serum 25-OHD concentrations for both cases and controls were highest in summer and autumn and lowest in winter. Though the season-matched mean serum 25-OHD levels were higher among controls compared to cases for all the seasons, the differences in means were not statistically significant. The highest differences in means were observed for spring (8.5 nmol/L) and were the lowest for summer (3.9 nmol/L) (Fig. 1).

Odds ratio comparing the fourth quartile of serum 25-OHD concentration to the first quartile was 0.63 (95% confidence interval (CI) 0.29–1.34, *P*-trend 0.10). Adjusting for age at first full-term pregnancy and region of residence, the odds ratio was OR 0.57 (95% CI 0.26–1.24, *P*-trend 0.07). When cases whose cancers were diagnosed within 1–3 years of serum sampling were excluded from the analysis, the odds ratio was slightly reduced and the trend test reached statistical significance, OR 0.43 (95% CI 0.18–1.05, *P*-trend 0.02) (Table 2). Women with sufficient 25-OHD concentrations (>75 nmol/L) had a significantly reduced risk of ovarian cancer compared to those with insufficient serum concentrations (OR 0.32; 95% CI 0.12–0.91, *p*-value 0.03). The number of women with sufficient serum 25-OHD concentrations were, however, small (20 women, 5 cases and 15 controls). The results were identical when season-defined quartiles were used.

Increasing serum calcium concentration was also inversely associated with ovarian cancer risk. Comparing the highest to the lowest quartile, the odds ratios were 0.46 (95% CI 0.23–0.95, *P*-trend 0.005) and 0.41 (95% CI 0.19–0.85, *P*-trend 0.004) in the first and multivariate adjusted models, respectively. Exclusion of cases diagnosed within 1–3 years of serum sampling had no material effect on the point estimate; OR 0.37 (95% CI 0.16-0.85, *P*-trend 0.002) (Table 3).

Compared to women who had low calcium/low 25-OHD concentrations, those who had high calcium/high 25-OHD levels had an odds ratio of 0.26 (95% CI 0.07–0.90). Likewise, the odds ratios among women with high calcium/low 25-OHD and low calcium/high 25-OHD were OR 0.41 (95% CI 0.19–0.87) and 0.51 (95% CI 0.29–1.05), respectively (see Table 4). Calcium does not modify the effect of 25-OHD ($p = 0.25$) and 25-OHD does not modify the effect of calcium ($p = 0.12$). The interaction is multiplicative.

4. Discussion

To the best of our knowledge, this is the first prospective study to examine the association of both serum calcium and 25-OHD with the risk of ovarian cancer. Our findings (i)

Table 1 – Baseline characteristics of ovarian cancer cases and matched, incidence density sampled controls who donated serum samples to the Finnish Maternity Cohort between 1983 and 2007.

	Cases (n = 172)	Controls (n = 172)
Age at serum sampling, years	29.9 (4.3)	29.9 (4.3)
Age at first full-term pregnancy, years	26.9 (4.3)	27.4 (4.3)
Age at last full-term pregnancy, years	31.4 (4.1)	32.1 (4.6)
Age at cancer diagnosis, years	36.4 (5.1)	
Lag time to cancer diagnosis, years (minimum, maximum)	6.4 (1, 13.5)	
Number of pregnancies	2.5 (1.0)	2.5 (1.0)
Gestational day at blood donation, d	76.6 (21.3)	78.9 (27.7)
25-OHD concentration nmol/L, median (10th, 90th percentile)	39.2 (23.8, 65.4)	40.0 (24.6, 73.4)
Calcium concentration mmol/L, median (10th, 90th percentile)	2.3 (1.9, 2.6)	2.4 (2.1, 2.8)

All values are expressed as mean (standard deviation) unless otherwise stated.

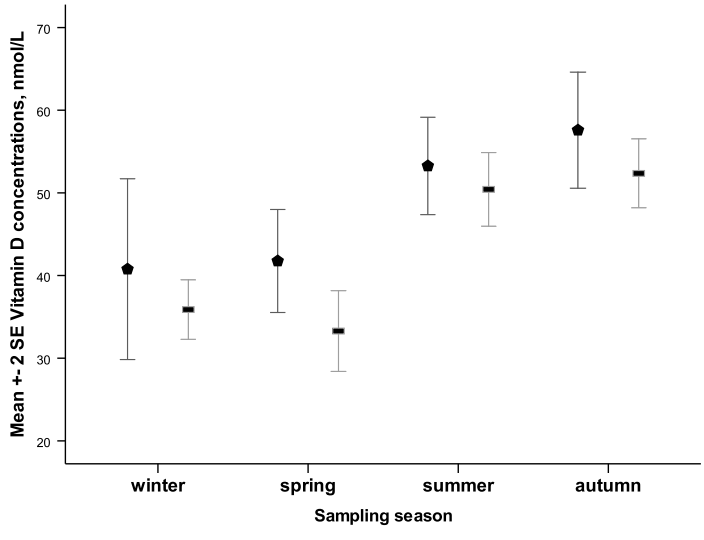

Fig. 1 – Mean serum vitamin D concentration (±2 SE) by season of blood donation among cases and controls.

Table 2 – Relative risk (estimated as odds ratio, OR, with 95% confidence intervals, CI) of ovarian cancer by quartile of serum 25-hydroxyvitamin D concentrations among Finnish women followed up to 13 years after sample donation.

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P-trend	Q4 versus Q1–3
Quartile values, nmol/L	<31.5	31.5–40.0	40.1–57.7	⩾57.8		
n, cases/control[a]	42/43	44/44	52/42	30/43		
OR	1.0 (reference)	1.05 (0.58–1.92)	1.25 (0.67–2.32)	0.63 (0.29–1.34)	0.10	0.56 (0.30–1.04)
OR, adjusted[b]	1.0 (reference)	1.01 (0.54–1.87)	1.13 (0.60–2.12)	0.57 (0.26–1.24)	0.07	0.54 (0.28–1.02)
Excluding cases who donated serum samples within 1–3 years of cancer diagnosis						
Quartile values	<32.0	32.1–40.5	40.6-58.1	⩾58.2		
n, cases/control	36/33	37/33	35/35	25/36		
OR, adjusted[b]	1.0 (reference)	0.93 (0.48–1.82)	0.79 (0.39–1.63)	0.43 (0.18–1.05)	0.02	0.49 (0.24–1.01)

[a] Controls were matched for age at serum sampling, parity and date of blood donation (±2 weeks).
[b] Adjusted for age at first full-term pregnancy and region of residence.

Table 3 – Relative risk (estimated as odds ratio, OR, with 95% confidence intervals, CI) of ovarian cancer by quartile of serum calcium concentrations among Finnish women followed up to 13 years after sample donation.

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P-trend
Quartile values, mmol/L	<2.2	2.2–2.4	2.4–2.6	⩾2.6	
n, cases/control[a]	46/39	50/45	48/44	26/44	
OR	1.0 (reference)	0.99 (0.54–1.85)	0.86 (0.46–1.60)	0.46 (0.23–0.95)	0.005
OR, adjusted[b]	1.0 (reference)	1.04 (0.55–1.96)	0.84 (0.44–1.61)	0.41 (0.19–0.85)	0.004
Excluding cases who donated serum samples within 3 years of cancer diagnosis					
Quartile values	<2.2	2.2–2.4	2.4–2.6		
n, cases/control	35/31	43/35	37/32	20/39	
OR, adjusted[b]	1.0 (reference)	1.26 (0.59–2.67)	0.97 (0.46–2.08)	0.37 (0.16–0.85)	0.002

[a] Controls were matched for age at serum sampling, parity and date of blood donation (±2 weeks).
[b] Adjusted for age at first full-term pregnancy and region of residence.

Table 4 – Joint effect of exposure of 25-hydroxyvitamin D and calcium on the relative risk (estimated as odds ratio, OR, with 95% confidence intervals, CI) of ovarian cancer among Finnish women.

	Group 1	Group 2	Group 3	Group 4
Case/control	116/96	26/32	21/33	5/11
OR, adjusted[a]	1.0 (reference)	0.51 (0.29–1.05)	0.41(0.19–0.87)	0.26 (0.07–0.90)

Low vitamin D/low calcium – Vit D (Q1, Q2, Q3) and calcium (Q1, Q2, Q3). High vitamin D/low calcium – Vit D (Q4) and calcium (Q1, Q2, Q3). Low vitamin D/high calcium – Vit D (Q1, Q2, Q3) and calcium (Q4). High vitamin D/high calcium– Vit D (Q4) + calcium (Q4).
[a] Adjusted for age at first full-term pregnancy and region of residence.

a strong inverse association between serum calcium concentration and ovarian cancer risk with and without high serum 25-OHD concentrations, (ii) a borderline inverse association between high serum 25-OHD concentrations and ovarian cancer risk, but (iii) no synergistic interaction between 25-OHD and calcium with regard to ovarian cancer risk.

4.1. *Calcium and ovarian cancer*

An inverse relationship between dietary calcium and ovarian cancer has been reported in some studies but not all.[12–17] However, serum calcium does not reflect dietary calcium intake as this relationship is influenced by vitamin D, parathyroid hormone (PTH) and other dietary factors.[18,22] The Norwegian study[18] observed no significant effect of dietary intake of calcium and vitamin D on serum calcium concentrations in women. Though the biological processes by which calcium may influence ovarian cancer are largely unknown, possible mechanisms include (i) the effects of calcium on apoptosis, cell growth and proliferation,[23,24] (ii) effects of the calcium receptor (CaR) on cell proliferation and differentiation[24,25] and (iii) effects of calcium on down-regulating PTH production.[26]

Calcium regulates many important steps in the apoptotic pathway from early signalling to chromatin cleavage but the precise molecular mechanisms involved are not clear.[23] The calcium receptor also regulates homeostasis in response to changes in extracellular calcium concentrations. It modulates the equilibrium between proliferation and differentiation in response to changes in extracellular calcium concentrations.[24,25] Loss of CaR-induced response to extracellular calcium has been observed in ovarian cancers.[25]

In hypocalcaemia, PTH stimulates the conversion of 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D in the kidneys. This leads to an increase in vitamin D availability which subsequently leads to increased calcium absorption and serum calcium.[26] The increase in serum calcium then provides a negative feedback causing a suppression of PTH production and reducing serum PTH levels. It has been hypothesised that PTH may be a tumour promoter acting as a co-mitogen and anti-apoptotic factor.[26] It also increases hepatic and osteoblastic synthesis of insulin-like growth factor-1 (IGF-1)[26,27] which has strong mitogenic effects and has been implicated in the pathogenesis of ovarian and other cancers.[28,29] The presence of growth regulating PTH receptors and PTH-related protein (PTH-rP) has been noted in cancer cells, including ovarian cancer.[30] However, to what extent the PTH-induced increases in IGF-1 concentrations alter the physiological balance of IGF-1 is unknown. Hence, by down-regulating PTH production, calcium potentially mitigates against the mitogenic and anti-apoptotic effects of PTH. This same hypothesis has also been proposed as one of the likely means by which vitamin D may protect against cancer,[26] but in our study, the protective effect of high calcium concentrations was seen irrespective of the vitamin D status.

4.2. *Vitamin D and ovarian cancer*

No previous study has observed a significant overall association between serum vitamin D and risk of ovarian cancer but borderline inverse associations have been reported in subgroup analyses.[5,6] We also observed a borderline inverse relationship between serum vitamin D and ovarian cancer in a secondary analysis excluding cases who donated samples

within 3 years prior to cancer diagnosis for which the trend test was statistically significant. However, there have been no previous reports of significant inverse association among women with sufficient serum vitamin D concentrations, a relative rarity in Finland, despite dietary fortification programmes since 1994. Previous studies by Tworoger et al. and us have observed borderline inverse associations among such groups.[5,6]

The present results differ slightly when compared to our earlier study within this cohort.[5] In that study, we conjectured that one of the reasons why no association was apparent could be because of the low serum 25-OHD within that cohort subset. This may hold true because the median serum 25-OHD among cases and controls in the present study is about 5 nmol/L higher than that of our earlier study. It also suggests that a minimum level of serum vitamin D is necessary to offer protection against ovarian cancer and in population groups where serum vitamin D concentrations are low; such protective effects may not be evident.

A recent large study observed a significant positive relationship between the number of FOK1 f alleles and ovarian cancer risk suggesting that the vitamin D pathway indeed plays a role in ovarian carcinogenesis,[31] but perhaps not in direct conjunction with calcium metabolism. Studies on the association between vitamin D receptor polymorphism and the risk of ovarian cancer have yielded conflicting results[31–33], indicating that the modes of action of vitamin D in the ovarian cancer context remain open.

4.3. *Joint effects of vitamin D and calcium in ovarian cancer*

We observed no evidence of a synergistic interaction between serum vitamin D and calcium on ovarian cancer risk. While calcium was independently associated with a reduced risk of ovarian cancer irrespective of vitamin D levels, vitamin D was independently associated with a non-significantly reduced risk of ovarian cancer. This suggests that the protective effects observed among women with high calcium and high vitamin D levels were likely to be mediated by the effects of high calcium levels. The concept of a possible synergistic effect of vitamin D and calcium on health outcomes has been proposed because of coherent observations in experimental and epidemiological studies. Calcium mediates vitamin D-induced apoptosis in breast cells[34] suggesting that parts of the apoptotic effects of vitamin D are made possible via calcium dependent processes. It has been shown that calcium deficiency promotes vitamin D deficiency and vitamin D requirements increased when dietary calcium reduces.[35] The randomised controlled trial conducted in United States also observed a reduced risk of total cancer among women on calcium supplementation but a more marked risk reduction among women on combined calcium + vitamin D supplementation. Likewise, signalling pathways for VDR and CaR converge on the same pathway giving rise to possibilities of interaction.[9]

4.4. *Methodological issues*

Our study has the following limitations. We did not have information on oral contraceptive use, family history of ovarian cancer and BMI which may influence our results; hence there may be residual confounding. It is however very likely that if the women were using oral contraceptives, they must have stopped for some months before the samples were was taken since they were pregnant women and the samples were taken between the 12th and 14th week of pregnancy. Also, our study subjects were limited to fertile women in their reproductive ages, hence we cannot generalise our results to infertile/and/or post-menopausal women.

The use of single-point biomarker measurement in determining risks may be questioned but studies have shown that there is a high individual correlation in 25-OHD concentrations over a 5 year period[36] and both 25-OHD and calcium exhibit low long-term intra-individual variations.[36,37] Likewise, we do not think pregnancy has affected the relationship between calcium, vitamin D and ovarian cancer because it has been shown that the serum calcium and 25-OHD concentrations are not appreciably different from the pre-pregnancy levels,[38] a result corroborated in our study whereby there was no correlation between gestational days and the two biomarkers.

In conclusion, calcium and vitamin D act independently to reduce ovarian cancer risk. While we observed a significant inverse association between calcium and ovarian cancer, the association between vitamin D and ovarian cancer was of borderline significance. The relationship between calcium and ovarian cancer is novel and intriguing, but needs to be validated in other studies as this could present new opportunities in ovarian cancer prevention.

Conflict of interest statement

None declared.

REFERENCES

1. Garland CF, Morh SB, Gorham ED, Grant WB, Garland FC. Role of ultraviolet B irradiance and vitamin D in prevention of ovarian cancer. *Am J Prev Med* 2006;**31**(6):512–4.
2. Grant WB. An ecological study of cancer mortality rates in Spain and with respect to indices of solar UVB irradiance and smoking. *Int J Cancer* 2006;**120**:1123–6.
3. Salazar-Martinez E, Lacano-Ponce EC, Gonzalez Lira-Lira G, Escudero-De los Rios P, Hernandez-Avila M. Nutritional determinants of epithelial ovarian cancer risk: a case–control study in Mexico. *Oncology* 2002;**63**:151–7.
4. Jiang F, Bao J, Li P, Nicosia SV, Bai W. Induction of ovarian cancer cell apoptosis by 1,25-dihydroxyvitamin D3 through down-regulation of telomerase. *J Biol Chem* 2004;**279**(51):53213–21.
5. Toriola AT, Surcel H-M, Agborsangaya C, et al. Serum 25-hydroxyvitamin D and the risk of ovarian cancer. *Eur J Cancer* 2010;**46**(2):364–9.
6. Tworoger SS, Lee I-M, Buring JE, Rosner B, Hollis BW, Hankinson SE. Plasma 25-hydroxyvitamin D and 1,25 dihydroxyvitamin D and the risk of incident ovarian cancer. *Cancer Epidemiol Biomarkers Prev* 2007;**16**:783–8.
7. Arslan AA, Clendenen TV, Koenig KL, et al. Circulating vitamin D and the risk of epithelial ovarian cancer. J Oncol 2009; doi:10.1155/2009/672492.

8. Heaney RP. Vitamin D and calcium interactions: functional outcomes. *Am J Clin Nutr* 2008;**88**(Suppl.):541S–4S.
9. Peterlik M, Grant WB, Cross HS. Calcium, vitamin D and cancer. *Anticancer Res* 2009;**29**(9):3687–98.
10. Lappe JM, Travers-Gustafson D, Davies KM, Recker RR, Heaney RP. Vitamin D and calcium supplementation reduces risk: results of a randomized trial. *Am J Clin Nutr* 2007;**85**:1586–91.
11. Cho E, Smith-Warner SA, Spiegelman D, et al. Dairy foods, calcium and colorectal cancer: a pooled analysis of 10 cohort studies. *J Natl Cancer Inst* 2004;**96**:1015–22.
12. Goodman MT, Wu AH, Tung KH, et al. Association of dairy products, lactose, and calcium with the risk of ovarian cancer. *Am J Epidemiol* 2002;**156**:148–57.
13. Bidoli E, La Vecchia C, Talamani R, et al. Micronutrients and ovarian cancer: a case–control study in Italy. *Ann Oncol* 2001;**12**:1589–93.
14. Koralek DO, Bertone-Johnson ER, Leitzmann MF, et al. Relationship between calcium, lactose, vitamin D, and dairy products and ovarian cancer. *Nutr Cancer* 2006;**56**(1):22–30.
15. Gerkinger JM, Hunter DJ, Spiegelman D, et al. Diary products and ovarian cancer: a pooled analysis of 12 cohort studies. *Cancer Epidemiol Biomarkers Prev* 2006;**15**:364–72.
16. Kushi LH, Mink PJ, Folsom AR, et al. Prospective study of diet on ovarian cancer. *Am J Epidemiol* 1999;**149**:21–31.
17. Park Y, Leitzmann MF, Subar AF, Hollenbeck A, Schatzkin A. Dairy food, calcium, and the risk of cancer in the NIH-AARP diet and health study. *Arch Intern Med* 2009;**169**(4):391–401.
18. Jorde R, Sundsfjord J, Bønaa KH. Determinants of serum calcium in men and women: the Tromso study. *Eur J Epidemiol* 2001;**17**(12):1117–23.
19. Koskela P, Antilla T, Bjørge T, et al. Chlamydia trachomatis infection as a risk factor for invasive cervical cancer. *Int J Cancer* 2000;**85**:35–9.
20. Teppo L, Pukkala E, Lehtonen M. Data quality and quality control of a population-based cancer registry. Experience in Finland. *Acta Oncol* 1994;**33**:365–9.
21. Hollick MF. Vitamin D deficiency. *N Eng J Med* 2007;**357**:266–81.
22. Nordin BC. Calcium requirement is a sliding scale. *Am J Clin Nutr* 2000;**71**:1381–3.
23. McConkey DJ, Orrenius S. The role of calcium in the regulation of apoptosis. *Biochem Biophys Res Commun* 1997;**239**:357–66.
24. Ramasamy I. Recent advances in physiological calcium homeostasis. *Clin Chem Lab Med* 2006;**44**(3):237–73.
25. Rodland KR. The role of the calcium-sensing receptor in cancer. *Cell Calcium* 2004;**35**:291–5.
26. McCarty MF. Parathyroid hormone may be a cancer promoter – an explanation for the decrease in cancer risk associated with ultraviolet light, calcium and vitamin D. *Med Hypotheses* 2000;**54**(3):475–82.
27. Coxam V, Davicco M-J, Durand D, et al. The influence of parathyroid hormone-related protein on hepatic IGF-1 production. *Acta Endocrinol* 1992;**126**:430–3.
28. Lukanova A, Lundin E, Toniola P, et al. Circulating levels of insulin-like growth factor-1 and risk of ovarian cancer. *Int J Cancer* 2002;**101**(6):549–54.
29. Renehan AG, Zwahlen M, Minder C, et al. Insulin-like growth factor (IGF)-1, IGF binding protein-3, and cancer risk: systematic review and meta-regression analysis. *Lancet* 2004;**363**(9418):346–53.
30. Broadus AE, Mangin M, Ikeda K, et al. Humoral hypercalcemia of cancer. Identification of a novel parathyroid hormone-like peptide. *N Engl J Med* 1988;**319**:556–63.
31. Tworoger SS, Gate MA, Lee I-M, et al. Polymorphisms in vitamin D receptor and risk of ovarian cancer in four studies. *Cancer Res* 2009;**69**(5):1885–91.
32. Clendenen TV, Arslan AA, Koenig KL, et al. Vitamin D receptor polymorphism and the risk of epithelial ovarian cancer. *Cancer Lett* 2008;**18**(260):209–15.
33. Lurie G, Wilkens LR, Thompson PJ, et al. Vitamin D receptor gene polymorphism and epithelial ovarian cancer. *Cancer Epidemiol Biomarkers Prev* 2007;**16**:2566–71.
34. Sergeev IN. Calcium as a mediator of 1,25-dihydroxyvitamin D3-induced apoptosis. *J Steroid Biochem* 2004;**89**:419–25.
35. Clements MR, Johnson L, Fraser DR. A new mechanism for induced vitamin D deficiency in calcium deprivation. *Nature* 1987;**325**:62–5.
36. HofmanN JN, Yu K, Horst RL, Hayes RB, Purdue MP. Long-term variation in serum 25-hydroxyvitamin D concentration among participants in the prostate, lung, colorectal and ovarian cancer screening trial. *Cancer Epidemiol Biomarkers Prev* 2010;**19**(4):927–31.
37. Gallagher SK, Johnson LK, Milne DB. Short-term and long-term variability of indices related to nutritional status. I: Ca, Cu, Fe, Mg, and Zn. *Clin Chem* 1989;**35**(3):369–73.
38. Kovacs CS, Kronenberg HM. Maternal–fetal calcium and bone metabolism during pregnancy, pueperium and lactation. *Endoc Rev* 1997;**18**(6):832–72.

IV

Submitted

Can over time vitamin D status predict ovarian cancer risk? A longitudinal nested case-control study

Adetunji T Toriola[1,2], Calypse B Agborsangaya[1,2], Helja-Marja Surcel[1] Kjell Grankvist[3], Eero Pukkala[2,4], Annekatrin Lukanova[5] , Matti Lehtinen[2]

[1]National Institute for Health and Welfare, Finland

[2]Tampere School of Public Health, University of Tampere, Finland

[3]Department of Medical Biosciences, Umeå University, Umeå, Sweden

[4]Finnish Cancer Registry, Institute for Statistical and Epidemiological Cancer Research, Helsinki, Finland

[5]Department of Cancer Epidemiology, German Cancer Research Centre, Heidelberg, Germany

Address for correspondence: Adetunji T Toriola, National Institute for Health and Welfare, Finland, PL 310, FIN-90101. Phone: +358 20610 6210

Fax: +358 206106251

Email: Adetunji.toriola@uta.fi

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Abstract:

We studied the association of over time vitamin D status with ovarian cancer risk in 90 matched case/control pairs who donated serial samples either during winter or summer seasons. Mean lag time between sample withdrawals was 2.6 years. Women whose serum 25-OHD concentrations were continuously above the summer season-specific median had a decreased risk of ovarian cancer compared to women with below median concentrations, OR 0.21 (95% CI 0.05-0.99). Women who consistently maintain high serum 25-OHD levels over many years may have lower risks of ovarian cancer.

Vitamin D status is inversely associated with ovarian cancer risk [1,2,3]. Same season levels of serum 25-OHD levels are reproducible over long time periods [4, 5, 6]. Thus, the use of one-point serum 25-OHD measurement is adequate in epidemiology but, the ethiopathogenetic impact of pre-diagnostic changes in vitamin D status should be elaborated.

We measured serum 25-OHD concentrations in samples donated over consecutive pregnancies before ovarian cancer diagnosis in the population-based Finnish Maternity Cohort (FMC), established by the National Institute for Health and Welfare (THL), Finland in 1983 [7]. Following an informed consent, first trimester serum samples are withdrawn at the municipal maternity care units to screen for intrauterine infections. The remaining sample (1-3 mL of serum) is stored at -25 ºC at the THL, Oulu. Since 1983, 750,000 women (> 98% of pregnant Finnish women) have donated samples to the cohort.

Incident ovarian cancer cases were identified by the population-based Finnish Cancer Registry (FCR). All cancer cases diagnosed in Finland since 1953 have been reported to the FCR, with practically no losses to follow-up [8]. Eligible cases were 172 women who had donated a minimum of two serum samples to the FMC, the second sample donated at least one year before cancer diagnosis and the first sample at least one year earlier.

Eligible controls (1:1) were women who were alive and free of cancer at the time of diagnosis of the index case, and had donated at least two serum samples during consecutive pregnancies to the FMC. The controls were matched for (i) age at sample withdrawal ± 1 year, (ii) parity, and (iii) date of index blood sampling ± 2 weeks for both sets of samples.

Eventually, only cases and controls who donated both serum samples within the same season were selected to avoid confounding due to differences in the sampling seasons. There were 53 case/control pairs (mean ages at the first and second serum samplings; 27.7/27.5 and 30.1/30.1 years) whose 1st and 2nd samples were donated during winter and 37 case/control pairs (mean ages at the first and second serum samplings; 27.7/27.8 and 30.5/30.3 years) whose 1st and 2nd samples were donated during summer. The average time difference between the 1st and the 2nd samplings was 2.6 years

The study was approved by the ethical committee of the National Institute for Health and Welfare, Finland.

Quantification of 25-OHD was performed at the Department of Medical Biosciences, University of Umeå, Sweden using a 25-OHD radioimmunoassay (IDS Ltd, Boldon, UK). The manufacturer stated a specificity (% cross-reactivity) of 100% for 25-OHD3, 75% for 25-OHD2, 100% for 24, 25-OH2D3, and less than 0.01% and 0.3% for cholecalciferol (D3), and ergocalciferol (D2), respectively. The within, between, and total coefficients of variation of the assay were 4.3%, 17.1%, and 17.7% at level 32.9 nmol/L, respectively.

We divided the women into four groups based on the median 25-OHD concentrations for both 1st and 2nd samples. (i) Women with 25-OHD concentrations below the season-specific median values during both 1st and 2nd sampling periods (reference category), (ii) Women with 25-OHD concentration below the median value during the 1st sampling period but above the median value during the 2nd sampling period, (iii) 25-OHD concentration above the median value during the 1st sampling period but below the median value during the 2nd sampling period, (iv) 25-OHD concentrations above the median values during the 1st and 2nd sampling periods.

Correlation between the 25-OHD concentrations was assessed with the Pearson correlation coefficient. Relative risk of ovarian cancer (odds ratio, OR with 95% confidence interval, CI) was calculated using the conditional logistic regression and adjusted for age at first full term pregnancy and region of residence (south, west, central, east and northern Finland). All statistical analyses were performed using SPSS 18 for windows (SPSS, Inc., Chicago, IL), assuming two-sided $p < 0.05$.

Among women who donated samples during winter, the mean 25-OHD concentrations for 1st and 2nd samples were 39.3 and 37.4 nmol/L, respectively while during summer the mean 25-OHD concentrations for 1st and 2nd samples were 51.5 and 52.0 nmol/L respectively. Correlation between the 1st and 2nd sample serum 25-OHD concentrations was highly significant for both summer combinations ($rs = 0.60$, p-value ≤ 0.001) and winter

combinations (rs = 0.39, p-value = 0.004) for the controls but not cases (r = 0.25 and 0.16 respectively (figure 1)

Among women who donated their samples during summer season, having both serum 25-OHD concentrations above the median values was associated with a reduced risk of ovarian cancer, OR 0.21 (95% CI 0.05-0.99) (table 1). A protective trend for vitamin D levels increasing over time was noted albeit with borderline statistical significance (p-trend = 0.06, table 1).

Our study suggests that high serum 25-OHD levels during summer season over a longer period of time, average of 2.7 years, was associated with an inverse risk of ovarian cancer. The correlation (r = 0.47) between samples donated during summer is very similar to that observed in the Norwegian study (between 0.42 and 0.52) [4] indicating good tracking of vitamin D status by serum 25-OHD levels. In this context, the lack of correlation between 1st and 2nd sample 25-0HD concentrations among the cases is noteworthy and suggest that ethiopathogenetically relevant changes may occur in women due to develop ovarian cancer. Experimentally, it has been shown that high levels of vitamin D are needed to protect against ovarian carcinogenesis [9]. At some stage, women developing ovarian cancer deviate from consistent long-term serum 25-OHD levels.

The generally low serum 25-OHD levels observed during winter are noteworthy. Solar ultraviolet radiation is a function of the solar zenith angles which are very large and oblique in winter resulting in lower ultraviolet rays compared to summer [10]. In essence, the UVB irradiation threshold of 18 mJ/cm^2 required to induce vitamin D production is not reached during winter in Finland and most people have vitamin D insufficiency at least half a year [10]. The ethiopathogenetic impact of failing to maintain appropriate vitamin D status over time and subsequent risk of ovarian cancer warrant further investigation.

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References:

1. Toriola AT, Surcel H-M, Agborsangaya C, et al. Independent and joint effects of serum 25-0HD and calcium on ovarian cancer risk: a prospective nested case-control study. Eur J Cancer (in press)
2. Zheng W, Danforth KN, Tworoger SS. Circulating 25-hydroxyvitamin D and risk of epithelial ovarian cancer: Cohort Consortium Vitamin D Pooling Project of Rarer Cancers. Am J Epidemiol. 2010;172:70-80
3. Tworoger SS, Lee I-M, Buring JE, et al. Plasma 25-hydroxyvitamin D and 1,25 dihydroxyvitamin D and the risk of incident ovarian cancer. Cancer Epidemiol Biomarkers Prev 2007;16: 783-8
4. Jorde R, Sneve M, Hutchinson M, et al. Tracking of serum 25-hydroxyvitamin D levels during 14 years in a population-based study and during 12 months in an intervention study. Am J Epidemiol 2010;171:903-8
5. Kostopoulos J, Tworoger SS, Campos H et al. Reproducibility of plasma and urine biomarkers among premenopausal and postmenopausal women from the Nurses Health Studies. Cancer Epidemiol Biomarkers Prev 2010;19:938-46
6. Agborsangaya C, Toriola AT, Grankvist K et al. The effects of storage time and sampling season on the stability serum 25-hydroxyvitamin D and androstenedione. Nutr Cancer 2010; 62:51-7
7. Koskela P, Antilla T, Bjørge T et al. Chlamydia trachomatis infection as a risk factor for invasive cervical cancer. Int J Cancer 2000;85:35–9
8. Teppo L, Pukkala E, Lehtonen M. Data quality and quality control of a population-based cancer registry. Experience in Finland. Acta Oncol 1994;33:365–9
9. Miettinen S, Ahonen MH, Lou YR et al. Role of 24-hydroxylase in vitamin D3 growth response of OVCAR-3 ovarian cancer cells. Int J Cancer 2004; 108: 367-73
10. Vähävihu K, Ylianttila L, Kautiainen H et al. Narrowband ultraviolet B course improves vitamin D balance in women in winter. Br J Dermatol 2010; 162:848-53
11. Kimlin MG. Geographical location and vitamin D synthesis. Mol Aspects Med 2008; 29:453-461

12. Matsuoka LY, Wortsman J, Haddad JG Hollis BW. In vivo threshold for cutaneous synthesis of vitamin D3. J Lab Clin Med 1989;114:301-5

Table 1. Relative risk (odds ratio, OR, with 95% confidence intervals, CI) of ovarian cancer by consecutively determined serum 25-OHD concentrations among women who donated at least two samples to the FMC between 1983 and 2006

Sampling seasons	Group 1	Group 2	Group 3	Group 4	P-trend	Group 4 vs others
Summer/Summer (N=74)						
cases/controls, n	15/18	5/11	4/4	13/4		
OR, adjusted[1]	1.0 (reference)	1.09 (0.32-3.67)	0.56 (0.11-2.88)	0.20 (0.04-1.05)	0.06	0.21 (0.05-0.99)
Winter/Winter (N=106)						
cases/controls, n	16/17	10/13	10/10	16/15		
OR, adjusted[1]	1.0 (reference)	1.50 (0.51-4.41)	1.24 (0.42-3.66)	0.91 (0.35-2.45)	0.93	0.81 (0.33-1.95)

[1]Adjusted for age at first full term pregnancy and region (altitude) of residence
[2]Group 1- 25-OHD concentrations below the season-specific median values in the 1st and 2nd samples
Group 2- 25-OHD concentration below the median value in the 1st sample but above the median value in the 2nd sample
Group 3- 25-OHD concentration above the median value in the 1st sample but below the median value in the 2nd sample
Group 4- 25-OHD concentrations were above the median values in the 1st and 2nd samples

First and second sample serum 25-OHD concentrations among cases and controls who donated serum samples 1 to 13 years before ovarian cancer diagnosis